

A Review of Depleted Uranium Biological Effects: *In vivo* Studies

**Alexandra C. Miller, PhD
Uniformed Services University
Armed Forces Radiobiology Research Institute**

The work presented represents the opinion of the author and is not the opinion of the U.S. Department of Defense or the U. S. Government.

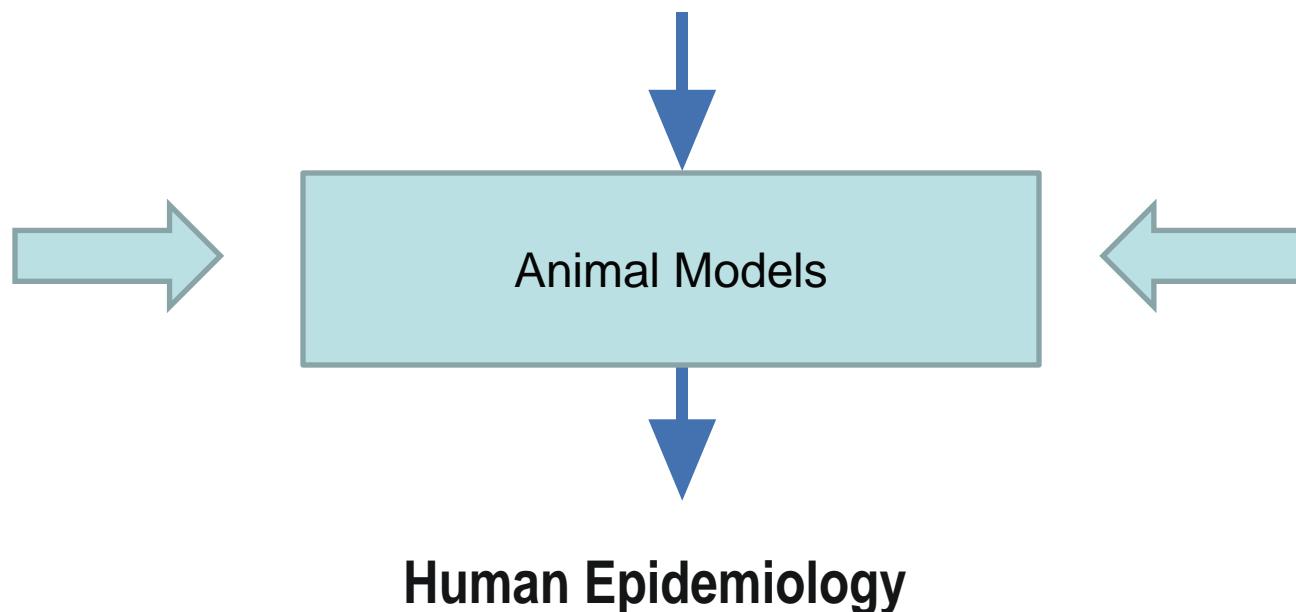
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Research Approach: Follow Regulatory Agency Approach

IARC, NTP, FDA, EPA

Carcinogenic Hazard Evaluation

Transformation + Mutagenicity + Cytogenicity



**"All models are wrong
but some are useful"**

Dr. George Box, Statistician, Mathematician

Problems in Evaluating Carcinogenicity

- 1) Epidemiology studies alone are not adequate – too few participants, confounders**
- 2) Animal studies alone are not adequate, relevance to humans, dose/dosing**
- 3) Cell studies alone are not adequate; relevance to humans,**

Examples:

- asbestos (animal studies ignored)
- saccharin (rodent carcinogen, not human)
- diethylstilbestrol (rodent model selection; ignored cell studies)

Questions Regarding DU And Its Health and Biological Effects that Prompted Our Research

- 1) Is long-term exposure to internalized DU toxic or carcinogenic?**
- 2) Does DU cause transgenerational effects?**
- 3) Does DU cause radiation effects?**
- 4) Can we distinguish between DU and other exposures (radiation, chemical)?**

Toxicology *in vivo*
DU Chronic Internal Exposure
via Implanted DU

- Sprague-Dawley Rat
- Implanted DU Pellets in Hind Limbs
- DU Pellet: 1mm diameter, 2 mm long
- Serial Euthanasia 6, 12, 18 months

In Vivo Model

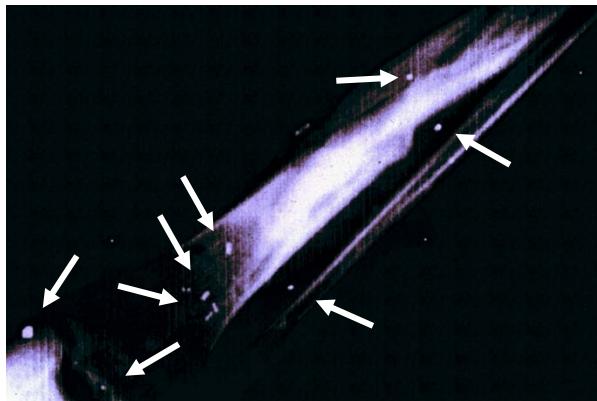
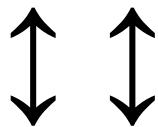
Implantation of DU Pellet



Relationship of Rodent DU Implants To Human DU Exposure (Wounding)



High Dose
20 or 6 pellets



Highest DU Excretor

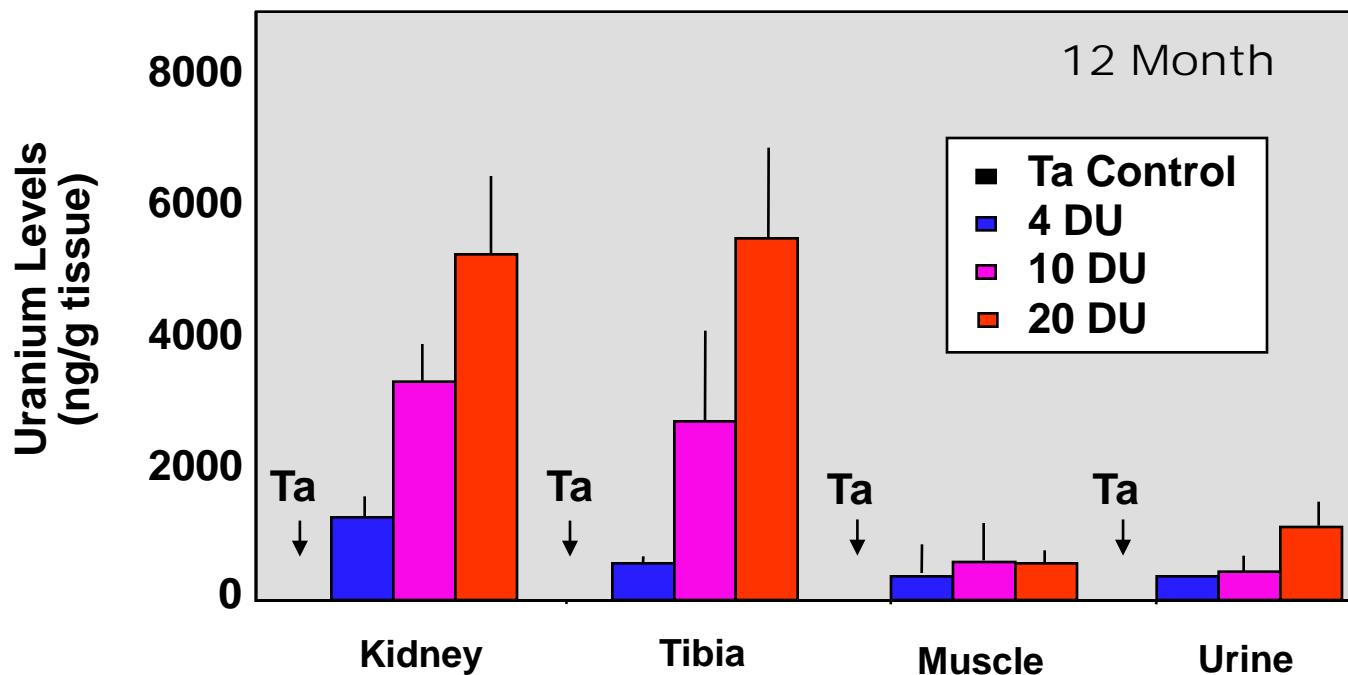


Health Effects of Embedded Depleted Uranium



*DU Pellet Implants
New and at 90 days*

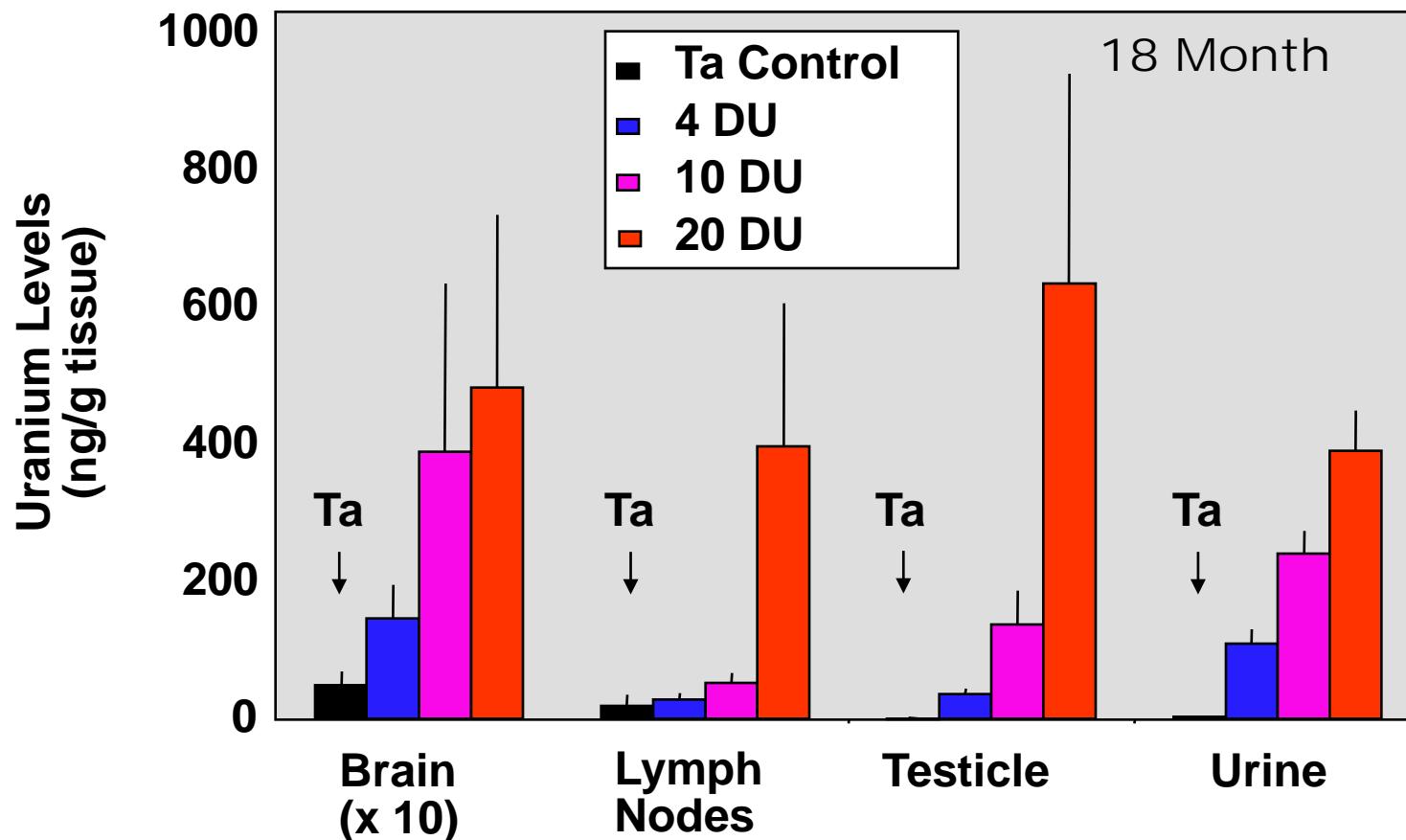
Uranium Distribution in Rat Tissue after DU Pellet Implantation



- ***Uranium redistributes with time to various organs and tissues, especially bone and kidney***
- ***No apparent changes in bone histology***

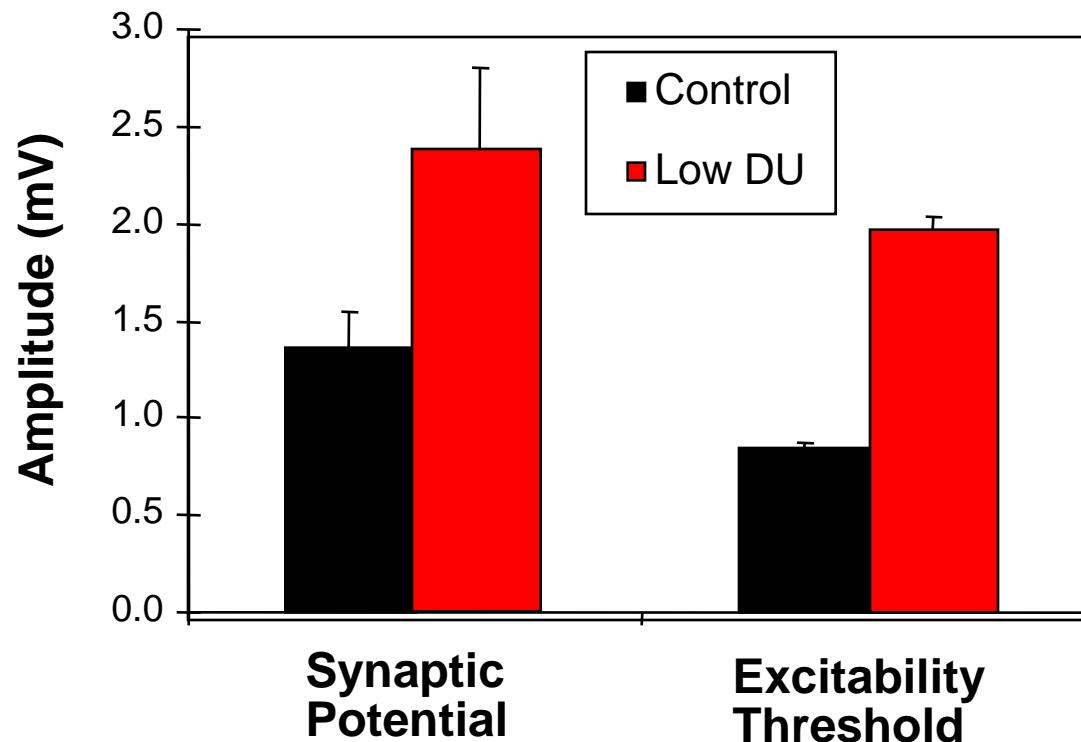
Pellmar et al., *Toxicol. Sci.* 49, 29-39 (1999)

Uranium Distribution in Rat Tissue after DU Pellet Implantation



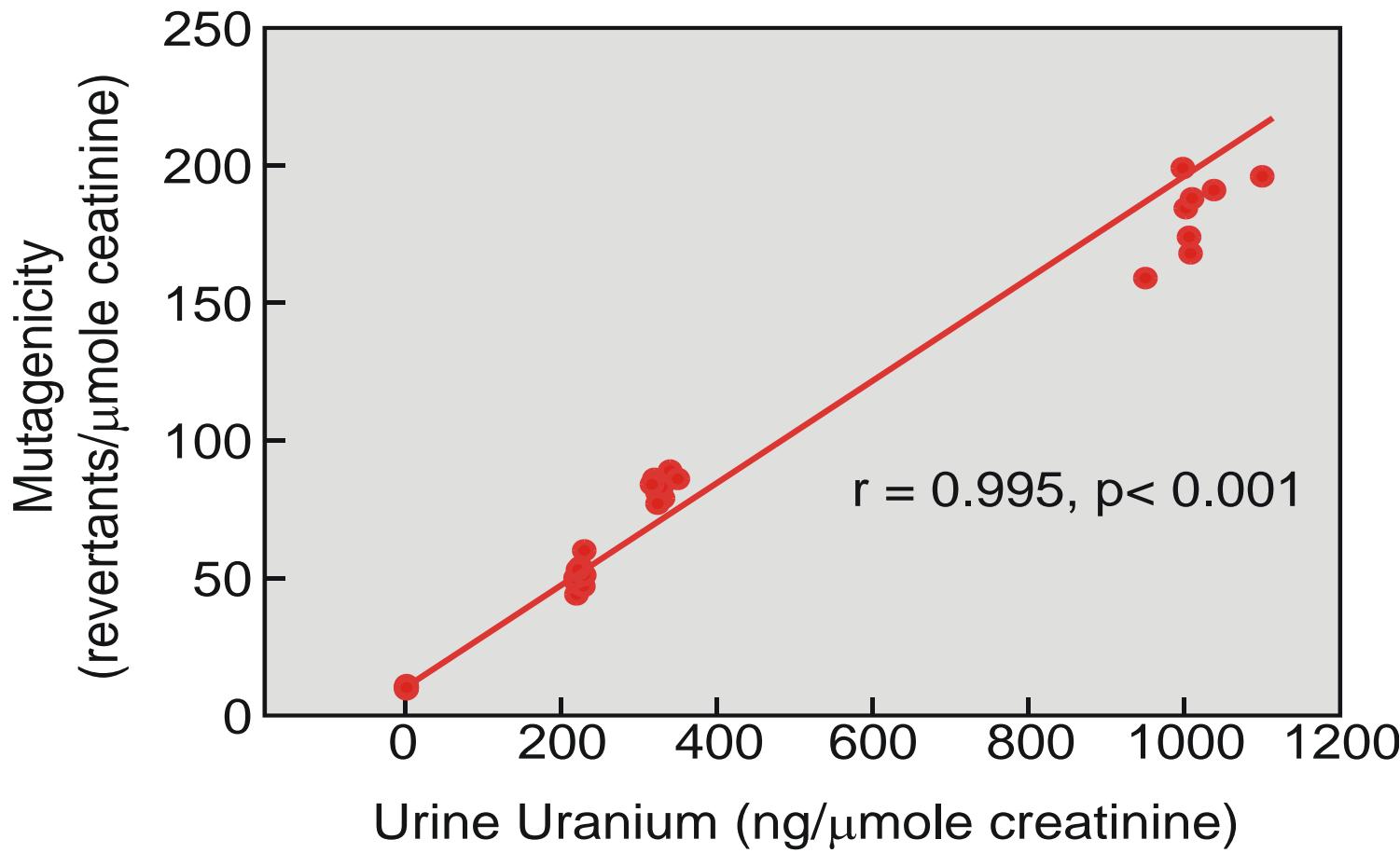
Neurotoxicity

Neurotoxicity 1 Year After DU Pellet Implantation



Mutagenicity

Correlation Between Urinary Mutagenicity
and Urinary Uranium Content
(12 Months Post DU Implantation)



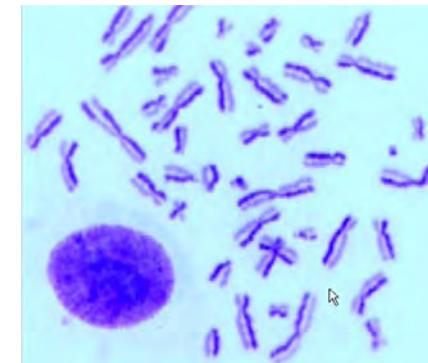
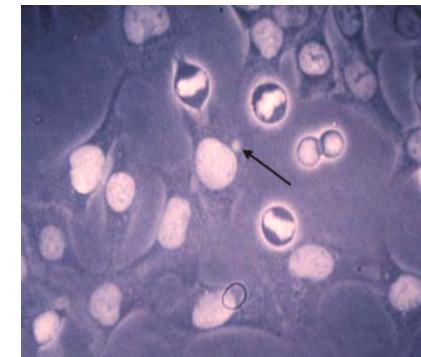
Miller *et al.*, *Mutagenesis*, vol., 13, no. 6, 643-648 (1998)

DU Genotoxicity *in vivo*

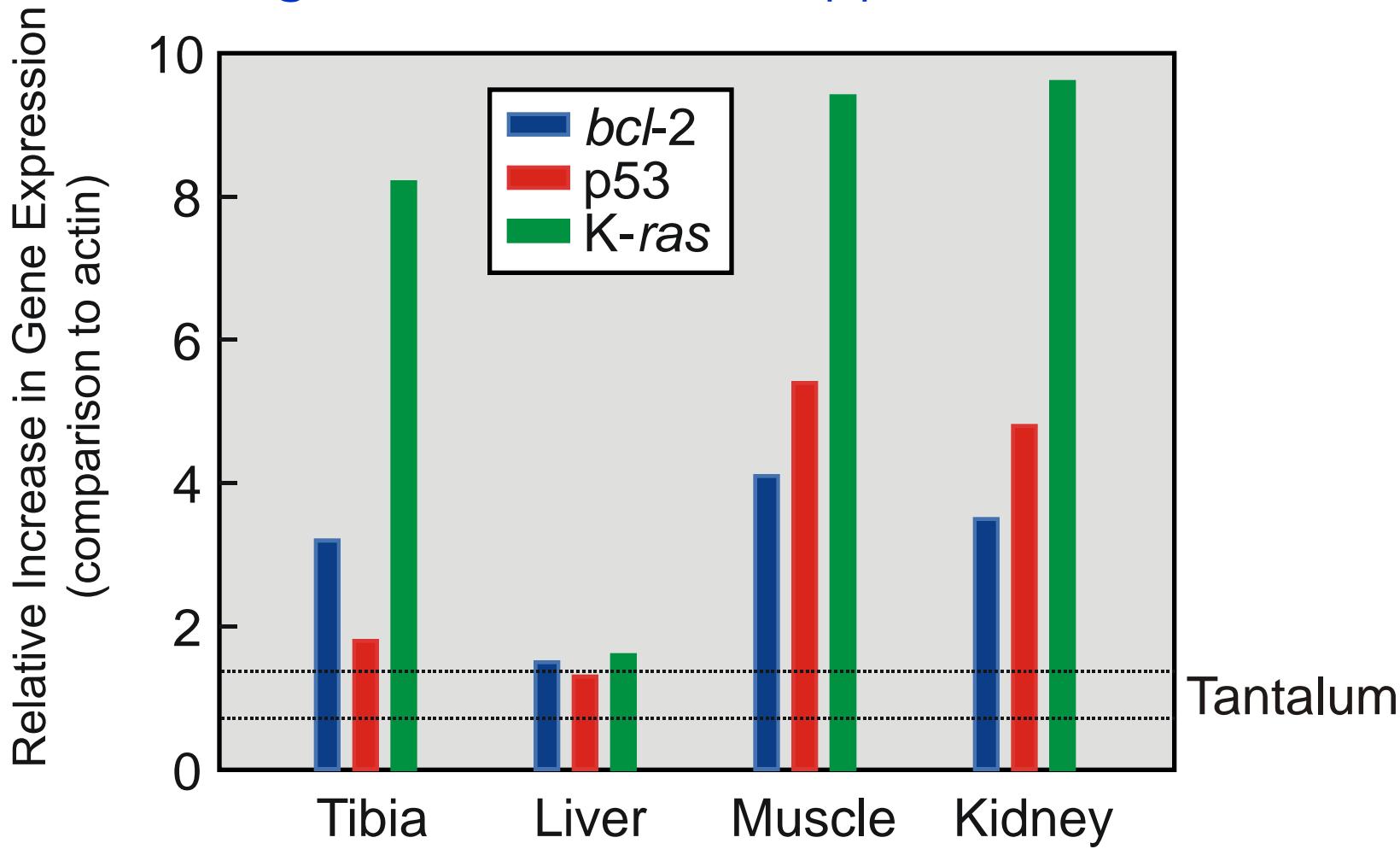
(Sprague-Dawley Rats)

Genotoxicity in DU-Implanted Rats
18 months post-implantation

Genotoxic Measure	Treatment	Frequency (%)	
Micronuclei	Control	0.31	0.03
	DU	0.55	0.05
	Ta	0.29	0.03
SCE	Control	0.40	0.04
	DU	0.91	0.08
	Ta	0.36	0.04
Chromosomal Aberrations	Control	0.25	0.03
	DU	0.49	0.05
	Ta	0.29	0.03



Genomic Effects of DU Oncogene and Tumor Suppressor Alterations



Conclusions from Toxicology Study of Embedded DU in Rat

- DU distributes to organs
- Uranium retention is long-term
- DU causes some neurotoxicity
- DU induces genotoxicity
- DU urine is mutagenic
- DU causes adverse oncogene changes

Toxicology *in vivo* DU Inhalation Studies

French Institute Nuclear Security
Pierrelatte, Fr
Fountenay au Roses, Fr

Genotoxic and Inflammatory Effects of Depleted Uranium Particles Inhaled by Rats

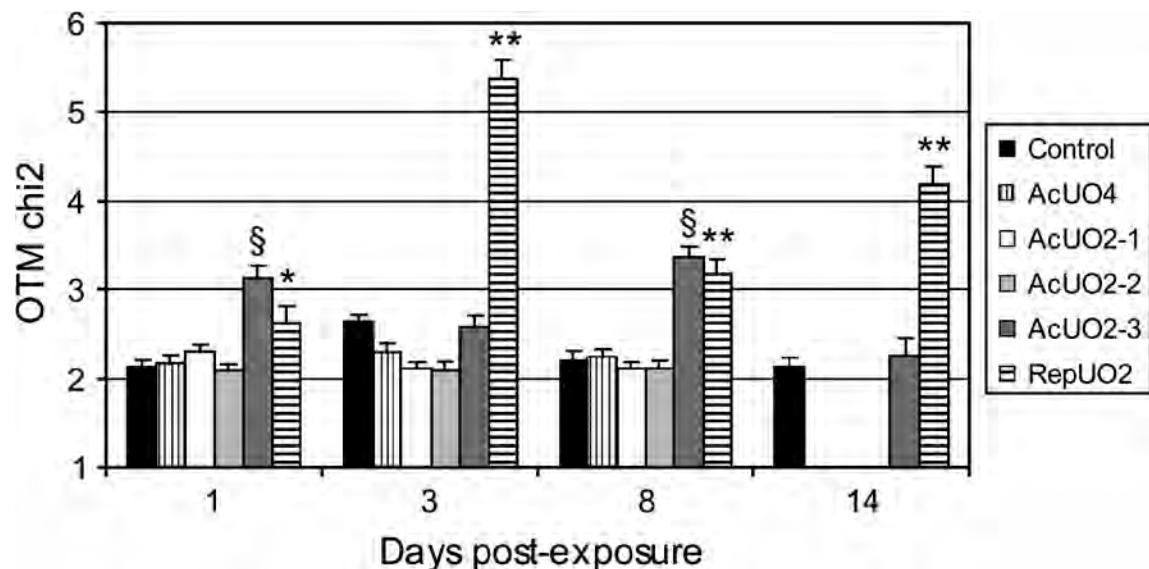
Experimental Protocol for Inhalation Study

- Note. h, hours; min, minutes.**

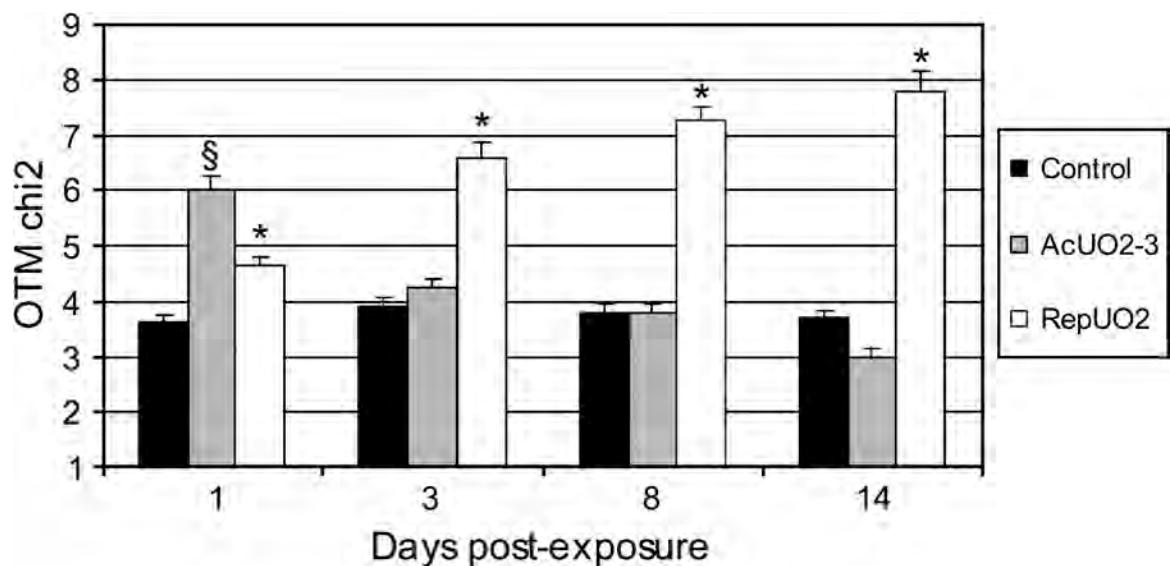
Inhalation type	Group name	Inhalation duration	Aerosols concentration	Euthanasia post-exposure
Acute UO ₂	AcUO2-1	30 min	190 mg.m ⁻³ ± 41 mg.m ⁻³	4 h, 1, 3, and 8 days
Acute UO ₂	AcUO2-2	2 h	375 mg.m ⁻³ ± 70 mg.m ⁻³	1, 3, and 8 days
Acute UO ₂	AcUO2-3	3 h	375 mg.m ⁻³ ± 70 mg.m ⁻³	1, 3, 8, and 14 days
Repeated UO ₂	RepUO2	30 min, 4 days/week, for 3 weeks	190 mg.m ⁻³ ± 41 mg.m ⁻³	1, 3, 8, and 14 days
Acute UO ₄	AcUO4	30 min	116 mg/m ³ ± 60 mg.m ⁻³	4 h, 1, 3, and 8 days
Air	Control	30 min, 4 days/week, for 3 weeks	Air	1, 3, 8, and 14 days

Monleau et al, 2006; **Toxicol Science**. Jan;89(1):287-95.

Genotoxic effects of depleted uranium particles inhaled by rats.

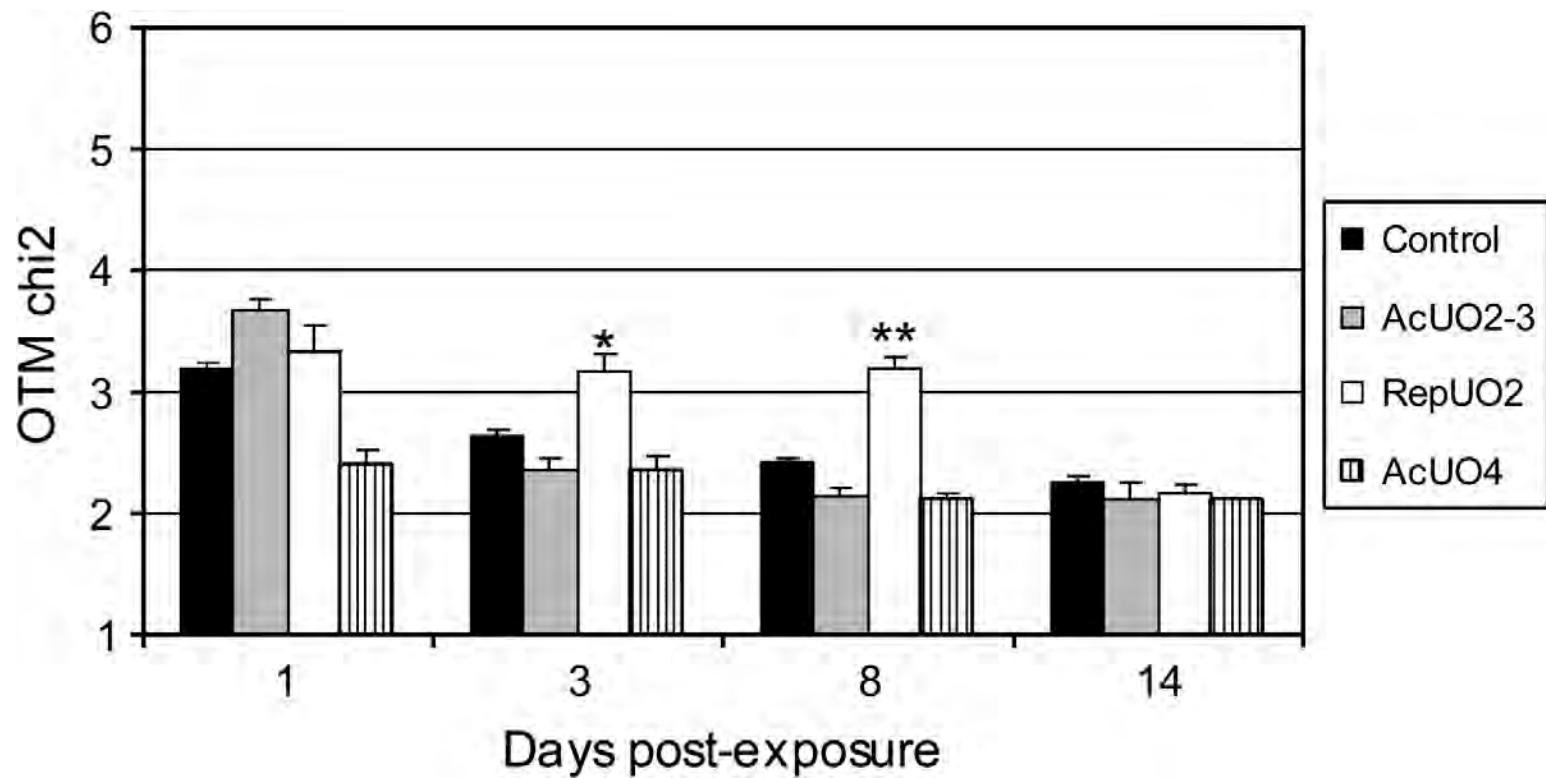


Comet assay in Bal cells
DNA strand breaks



Genotoxic effects of depleted uranium particles inhaled by rats.

Comet assay in Kidney cells - DNA strand breaks



Findings: Repeated Inhalations Most Genotoxic

DU Toxicology Studies: Oral Administration

Renal Anemia Induced by Chronic Ingestion of Depleted Uranium in Rats DU Effects on Hematological Parameters

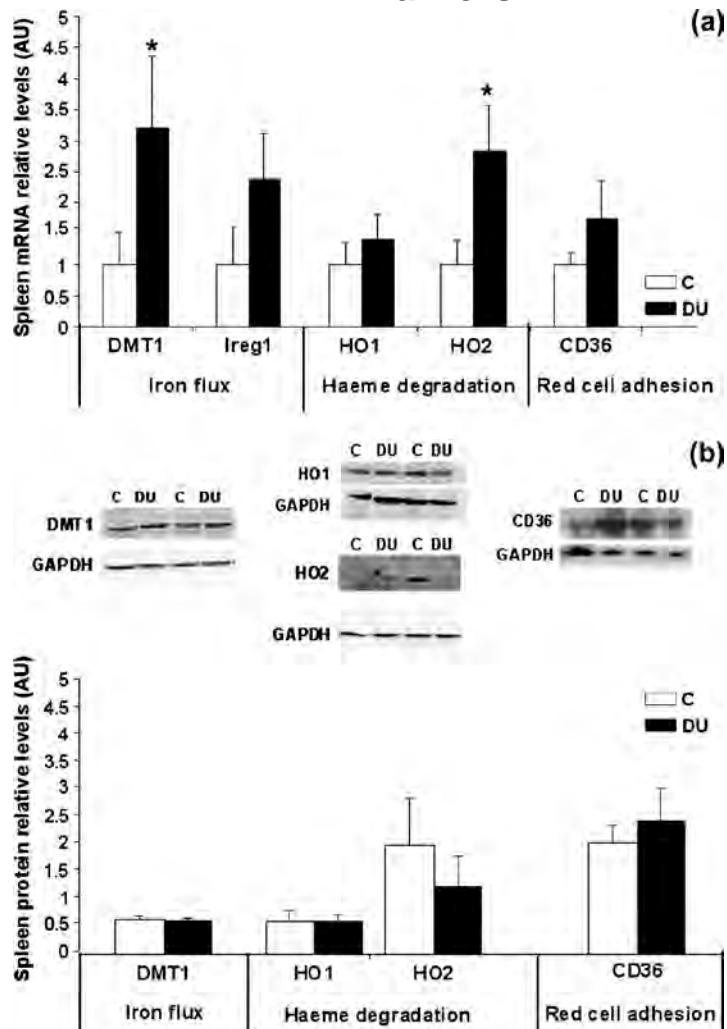
	Control (n = 7)	DU (n = 8)	p
Complete blood count			
RBC ($10^{12}/l$)	10.2 ± 0.5	8.3 ± 0.7	0.048*
Hemoglobin (g/l)	15.8 ± 1.8	12.8 ± 2.5	0.056
Hematocrit (%)	55.9 ± 3.1	45.5 ± 3.7	0.051
Leucocytes ($10^9/l$)	5.1 ± 0.9	5.2 ± 1.0	ns
Iron and binding capacity			
Iron concentration ($\mu\text{mol}/l$)	48.9 ± 7.4	39.5 ± 5.6	ns
UIBC ($\mu\text{mol}/l$)	90.2 ± 19.1	81.8 ± 8.6	ns
TIBC ($\mu\text{mol}/l$)	139 ± 15	121 ± 13	ns

Berradi H et al. *Toxicol. Sci.* 2008;103:397-408

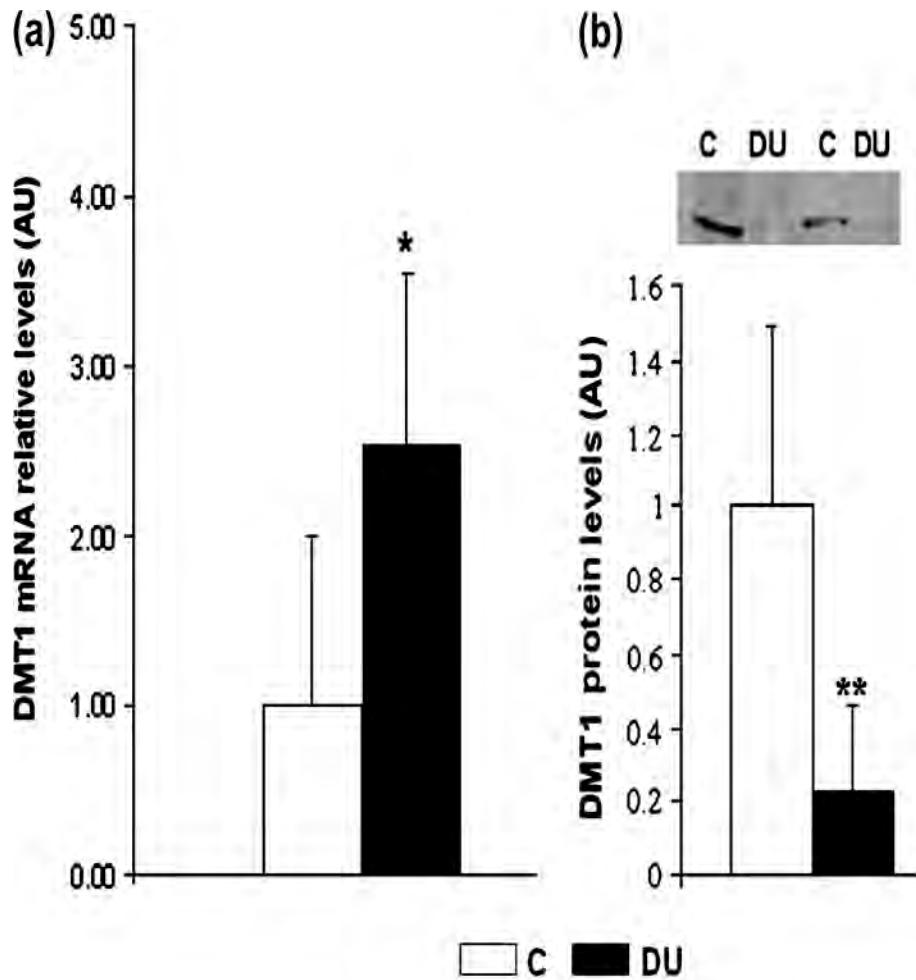
- Note. Statistical analyses were performed using Student t-test. *p < 0.05 significantly different from control group.
- A significant decrease was observed in RBC number.
- Hemoglobin and hematocrit also tended to diminish after chronic DU ingestion. ns, not significant.

Oral Administration of DU Induces Renal Anemia

Changes in expression of splenic iron recycling markers.



Renal expression of iron transporter DMT1 in DU-contaminated and control rats.



Summary

Evaluation of Oral or Inhaled DU Exposure on Rat Behavior, Toxicological Endpoints, Offspring Effects

Uranium Distribution	Behavior; Locomotor	Brain Accumulation	Sperm Effects	Immune Effects	Offspring Effects Behavior
Yes	Yes	Yes	No effects	Decreased Inflammatory pathways	F1 no cancers
Time and Dose Dependent	Time and Dose Dependent	Exposure type dependent		Decreased macrophages, mast cells	F1 ↑ hyperactivity

French Institute Nuclear Security (IRSN)

Muller M, et al., 2008, Uptake of Uranium, via endocytosis. *Toxicol Sci.* Feb;101(2):254-62

Dublineau I, et al, 2007, Modifications of Inflammatory pathways in intestine by DU . *Toxicol Sci.* 2007 Aug;98(2):458-68

Monleau, M, et al. 2006. Bioaccumulation and behavioural effects of depleted uranium in rats exposed to repeated inhalations.

Houpert P et al, 2005 Heterogeneous accumulation of uranium in the brain of rats. *Radiat Prot Dosimetry.* 2007;127(1-4):86-9.

Lestaev P, et al., 2005. Brain is a target organ for DU. *Toxicology.* Sep 1;212(2-3):219-26.

Questions Regarding DU And Its Health and Biological Effects that Prompted Our Research

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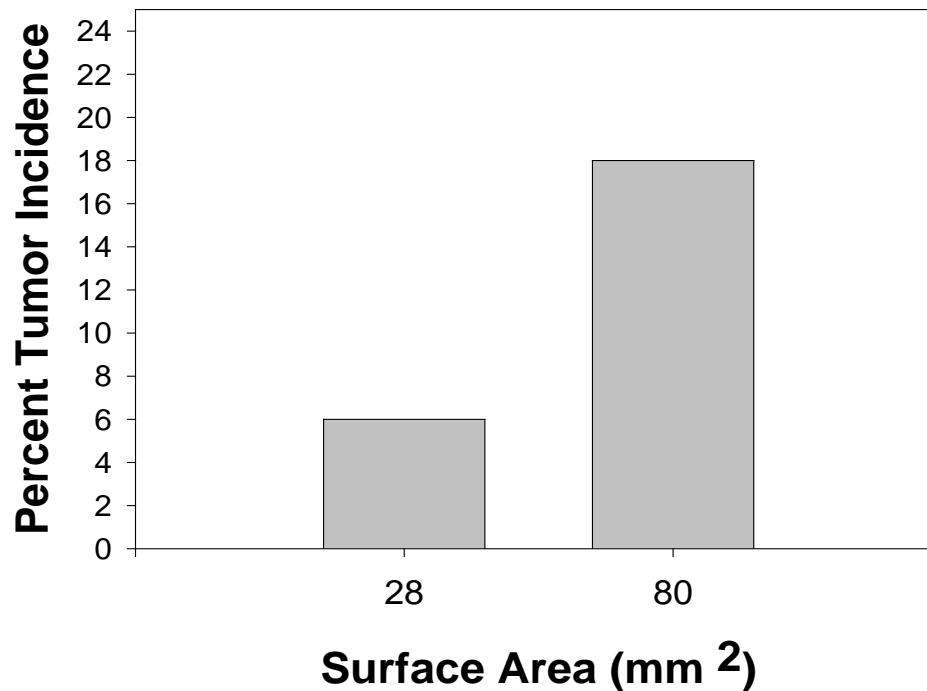
AFRRRI DU Carcinogenicity Study

Dr. Kalinich

DU Carcinogenicity

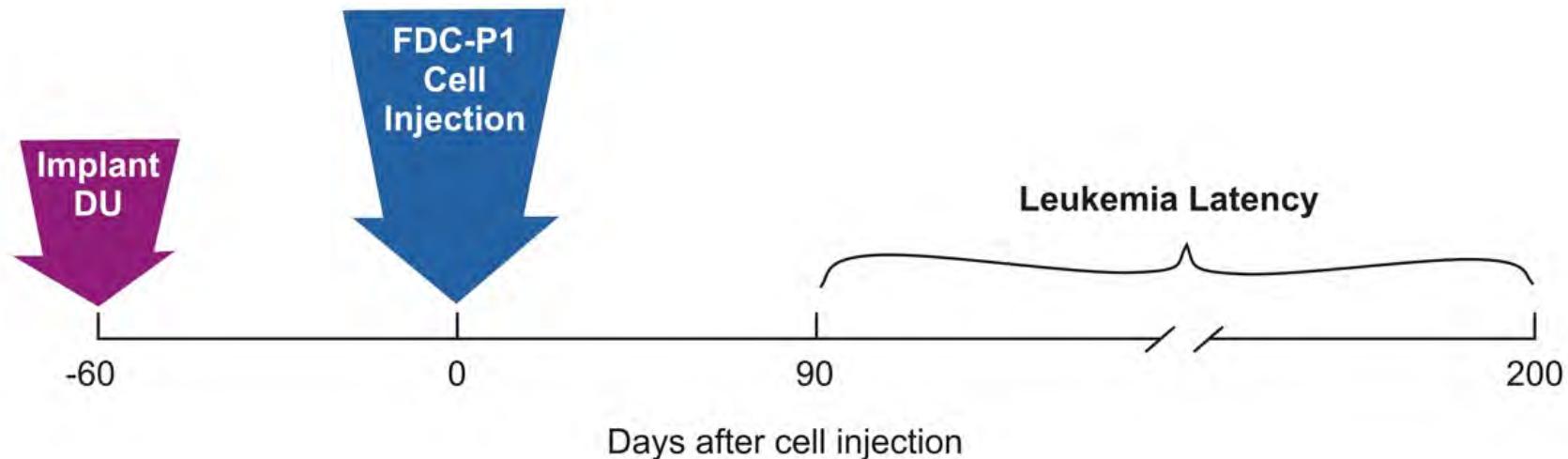
1995 MRMC-funded Study

Incidence of Soft Tumors in Rats Implanted with DU

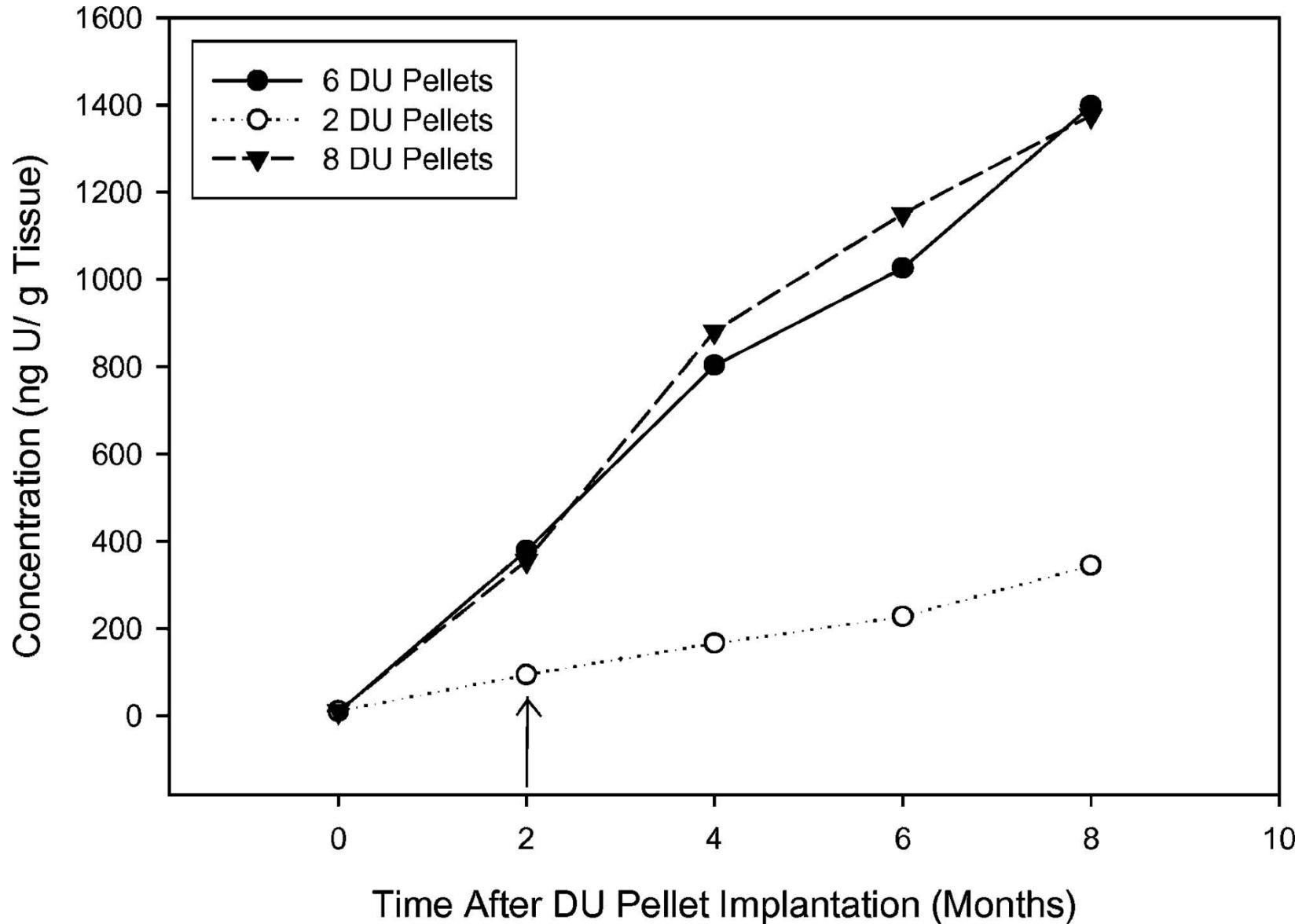


Hahn, et. al., *Environmental Health Perspectives*, 2002
Jan;110(1):51-9.

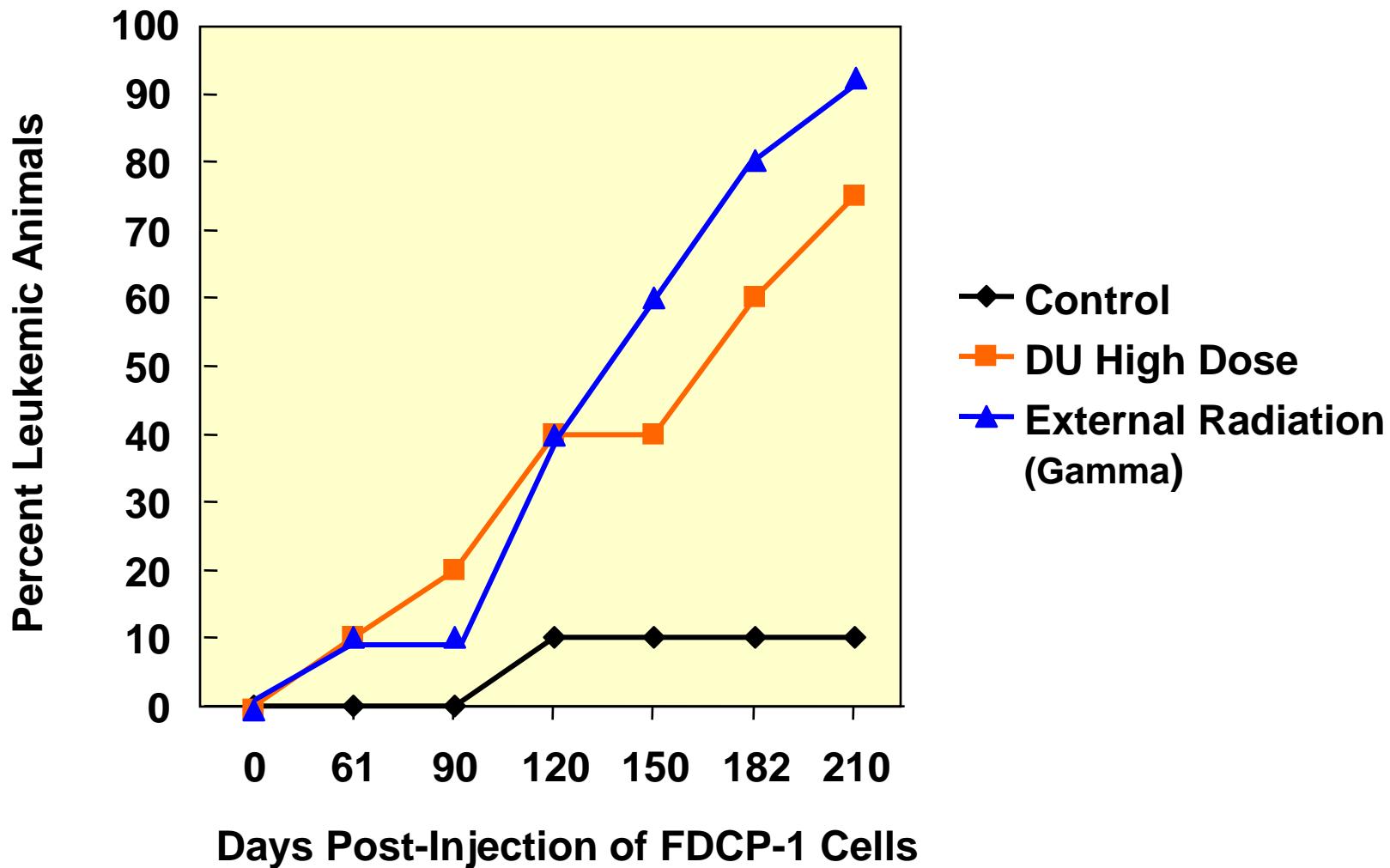
Model to Study Depleted Uranium-Induced Leukemia



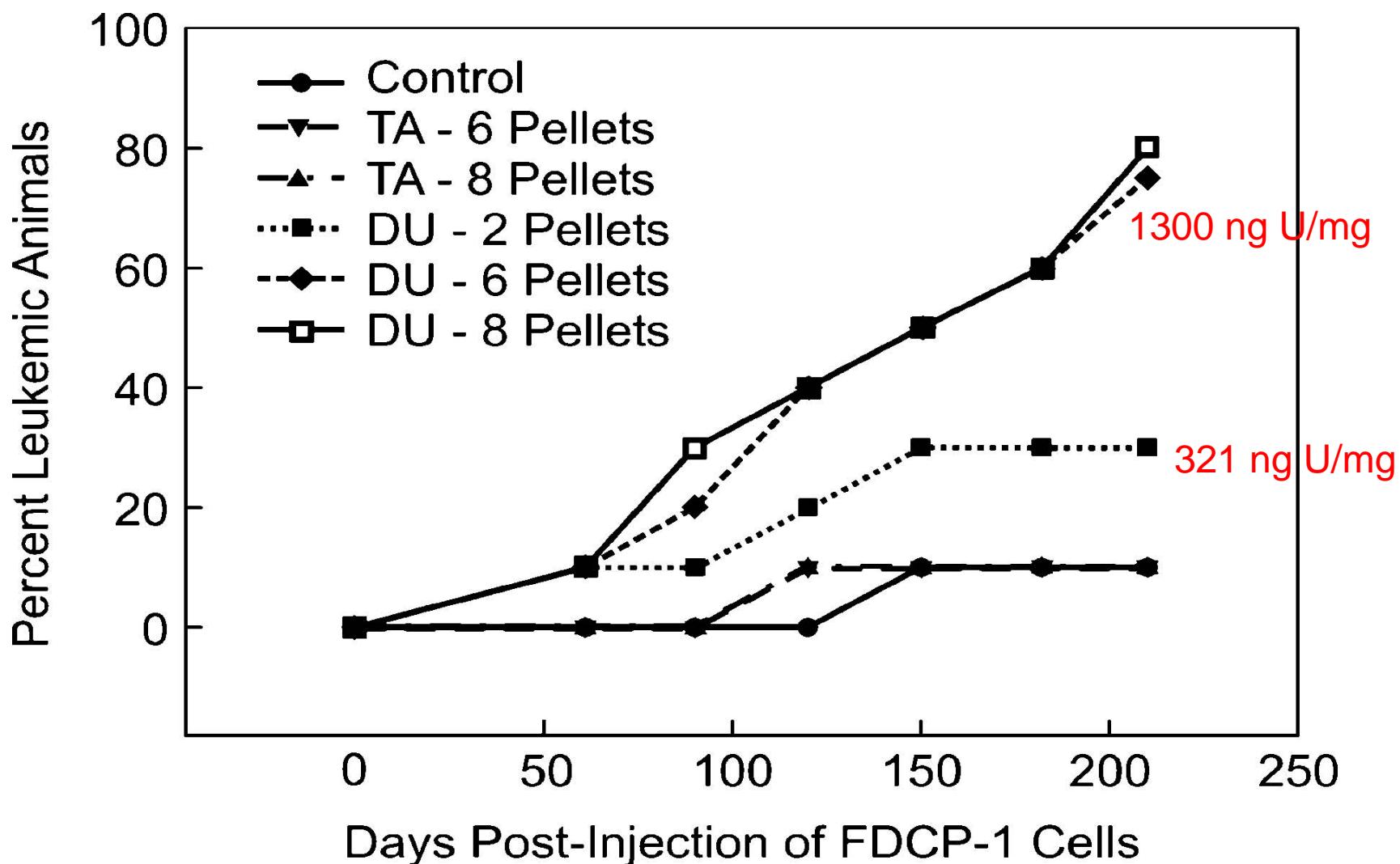
Bone Marrow Uranium Levels in DU-Exposed Mice



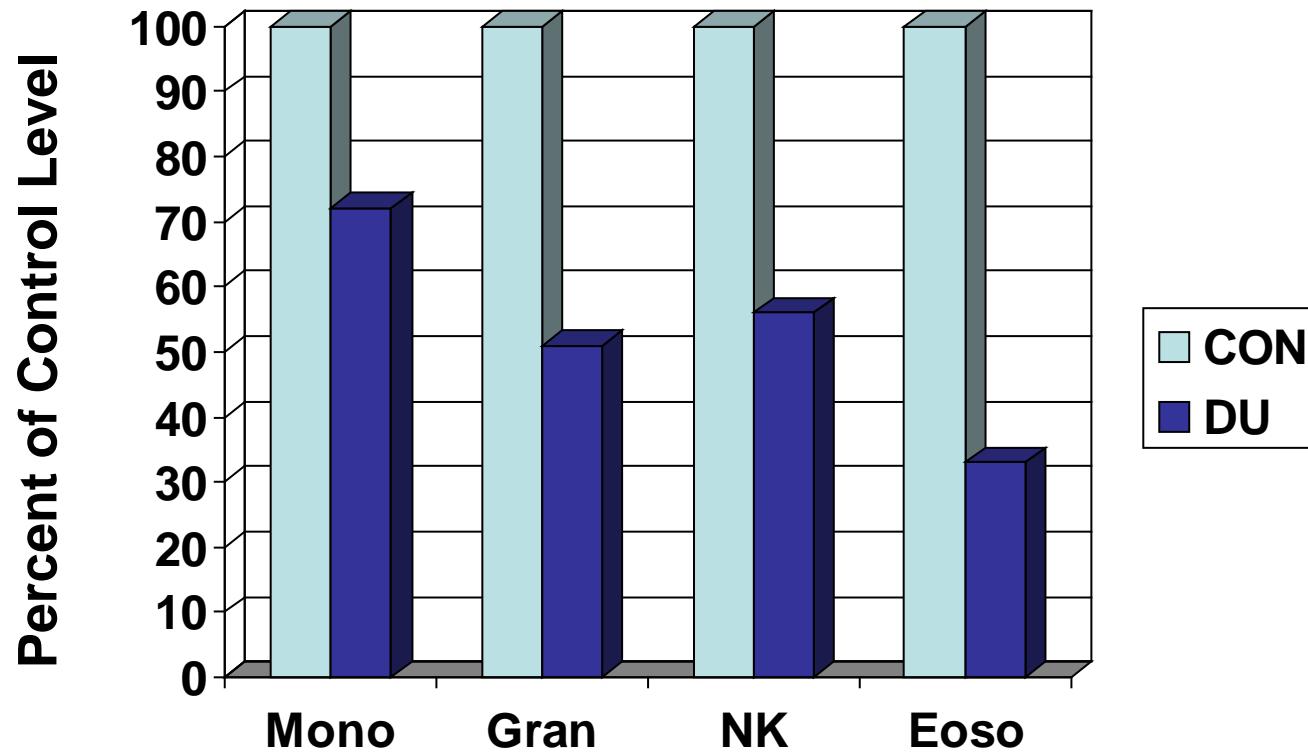
DU Carcinogenicity/Leukemogenesis *in vivo*: DU-Induced Leukemia after Exposure to Embedded DU for 60 Days



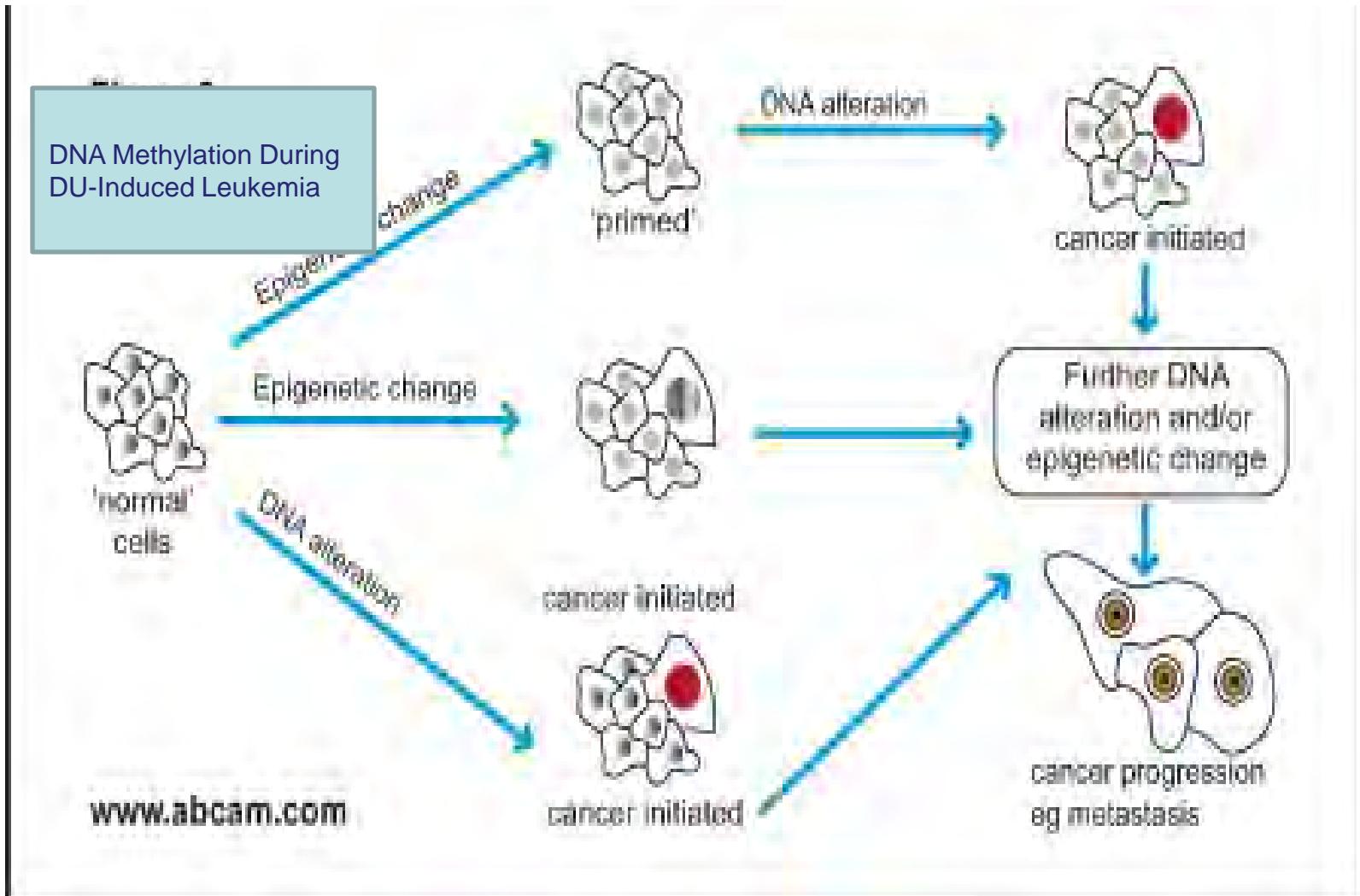
Incidence of DU-Dose on Leukemia Incidence Uranium Concentration-Dependent



Effect of DU on Blood Element Counts: 60 Days Post-Pellet Implantation



Mechanisms in Leukemia Induction "Genetic versus Epigenetic"

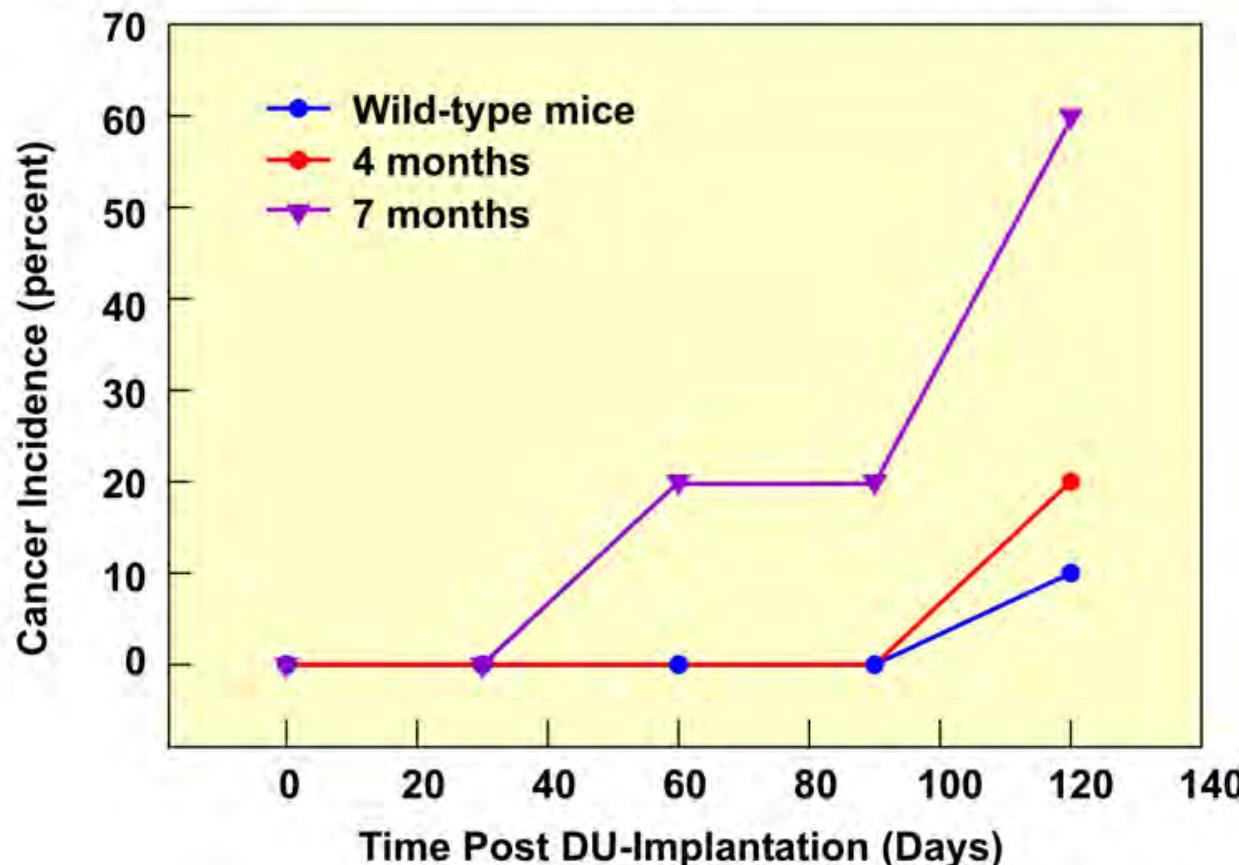


Finding: DU Induces Leukemia in a Mouse Model

Miller et al., *Biochimie* 91(11) 112-115, 2009.

Another Model: Bladder Carcinoma Model

**Urinary Bladder Lesions in DU-Implanted Mice:
Carcinoma *in situ* in *p53* deficient mice**



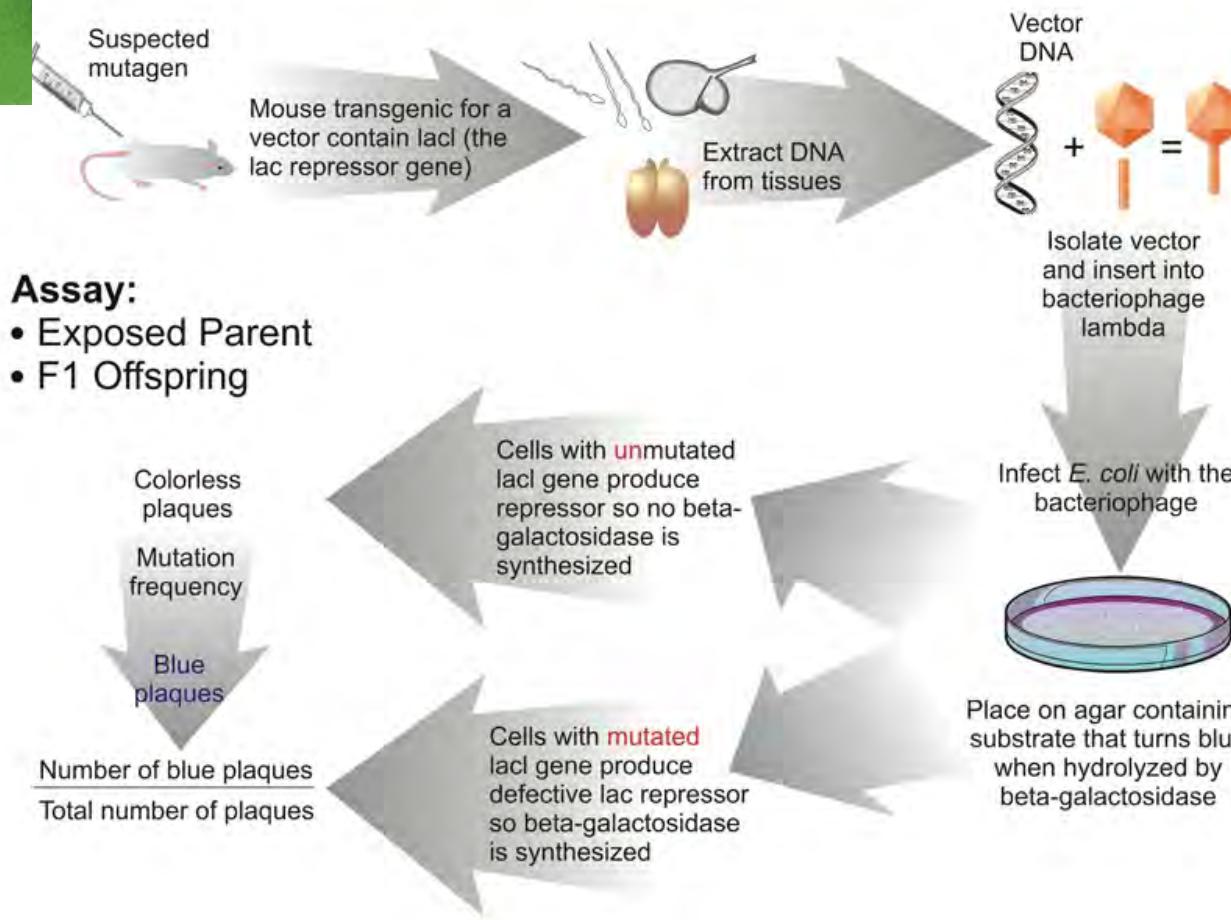
Finding: Preliminary

Questions Regarding DU And Its Health and Biological Effects that Prompted Our Research

- 1) Is long-term exposure to internalized DU carcinogenic?
- 2) Does DU cause transgenerational effects?
- 3) Does DU cause radiation effects?
- 4) Can we distinguish between DU and other exposures (radiation, chemical)?

Model to Assess Transgenerational Effects of Radiation or Heavy Metals

“Big Blue” Mutation and Offspring Assessment Assay



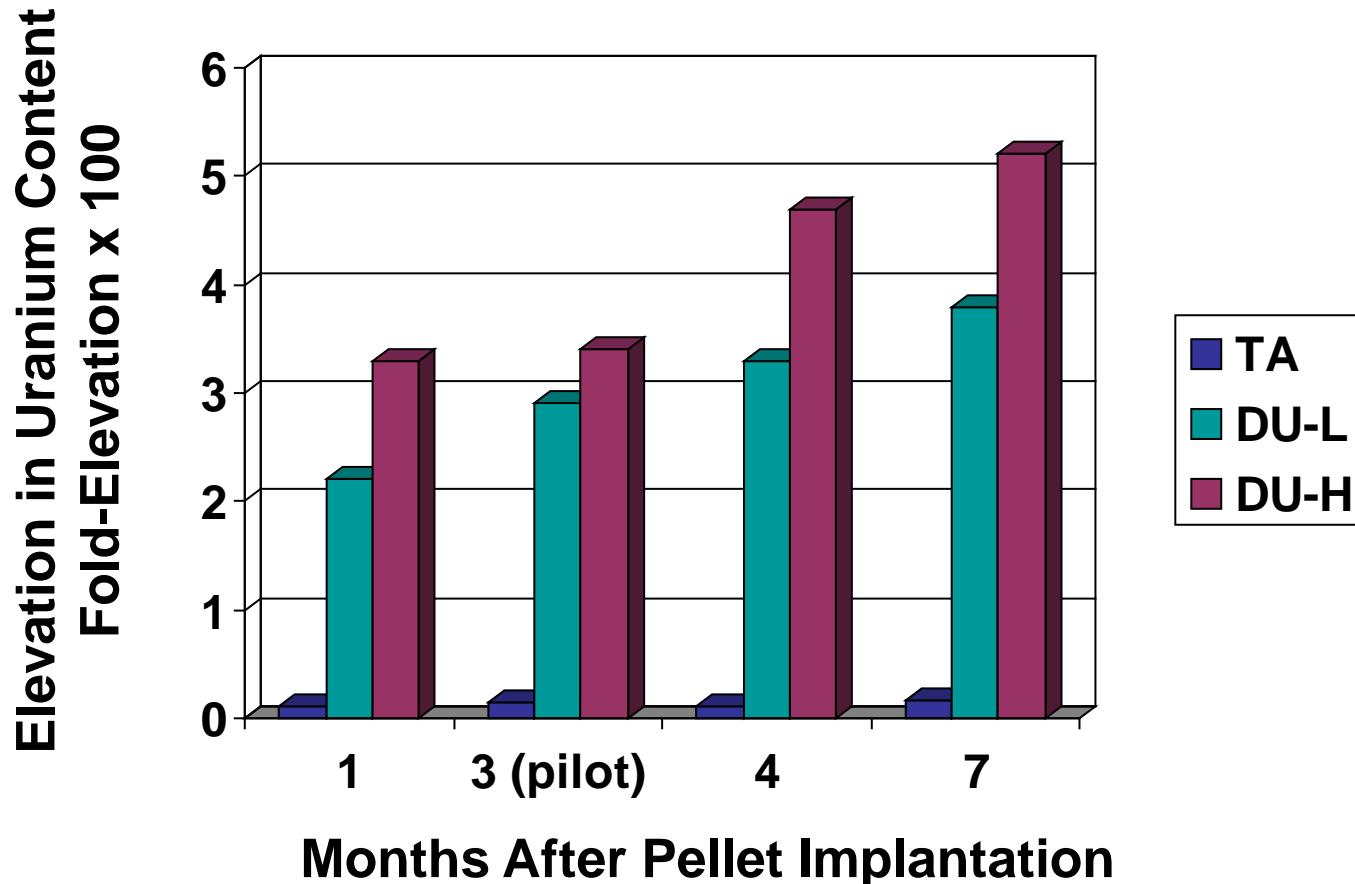
Implantation of DU Pellet



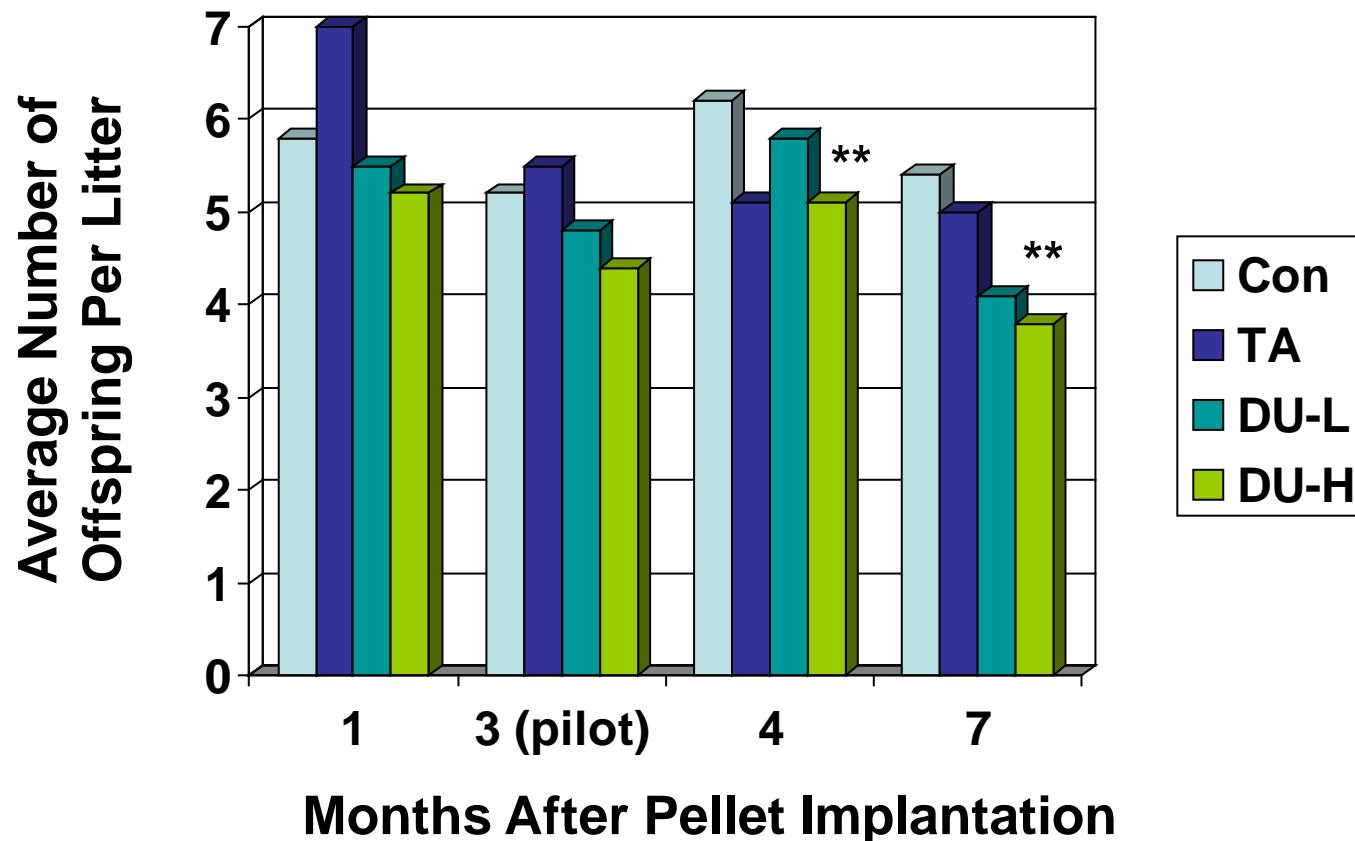
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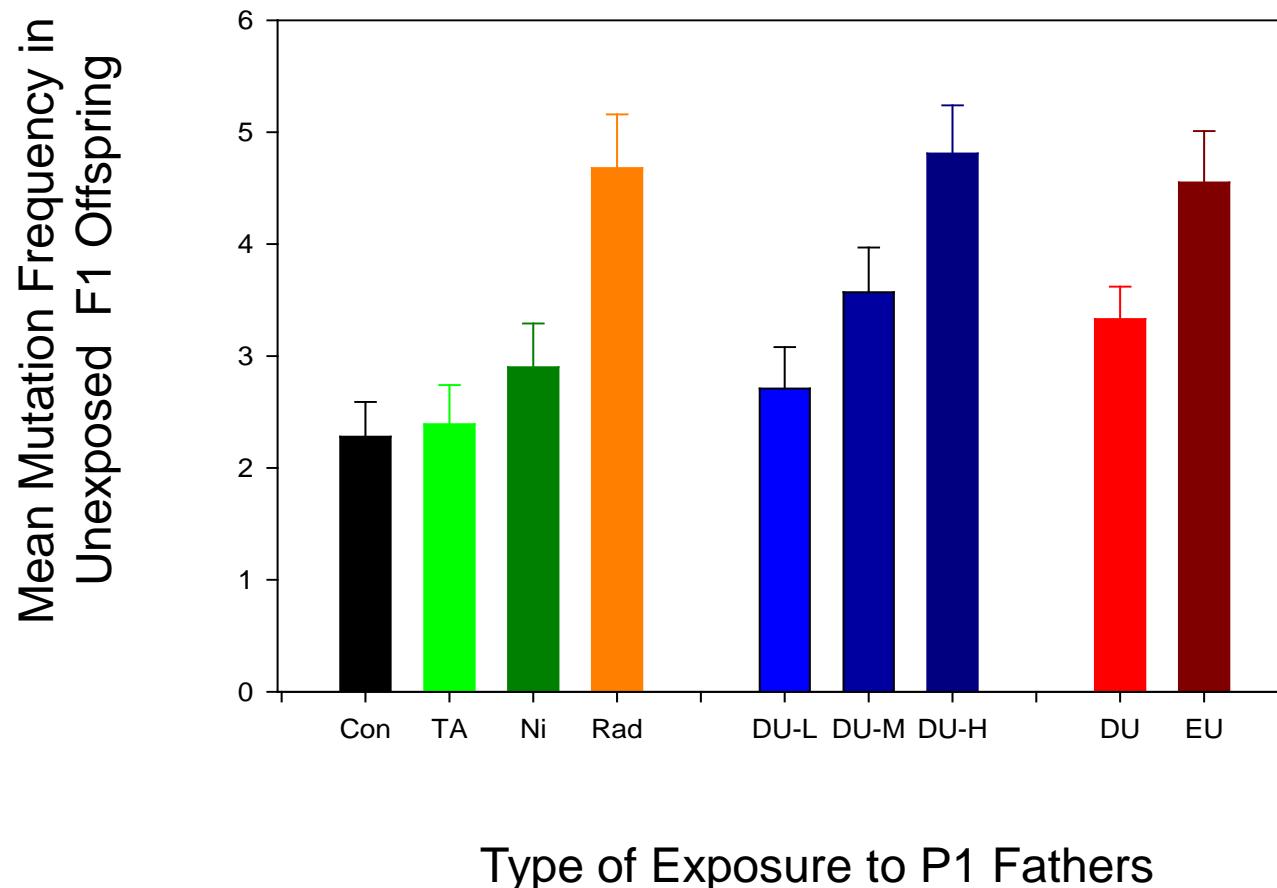
Effect of Paternal Exposure to DU on Testes Uranium Content



Effect of Paternal Preconceptional Exposure to DU on Litter Size

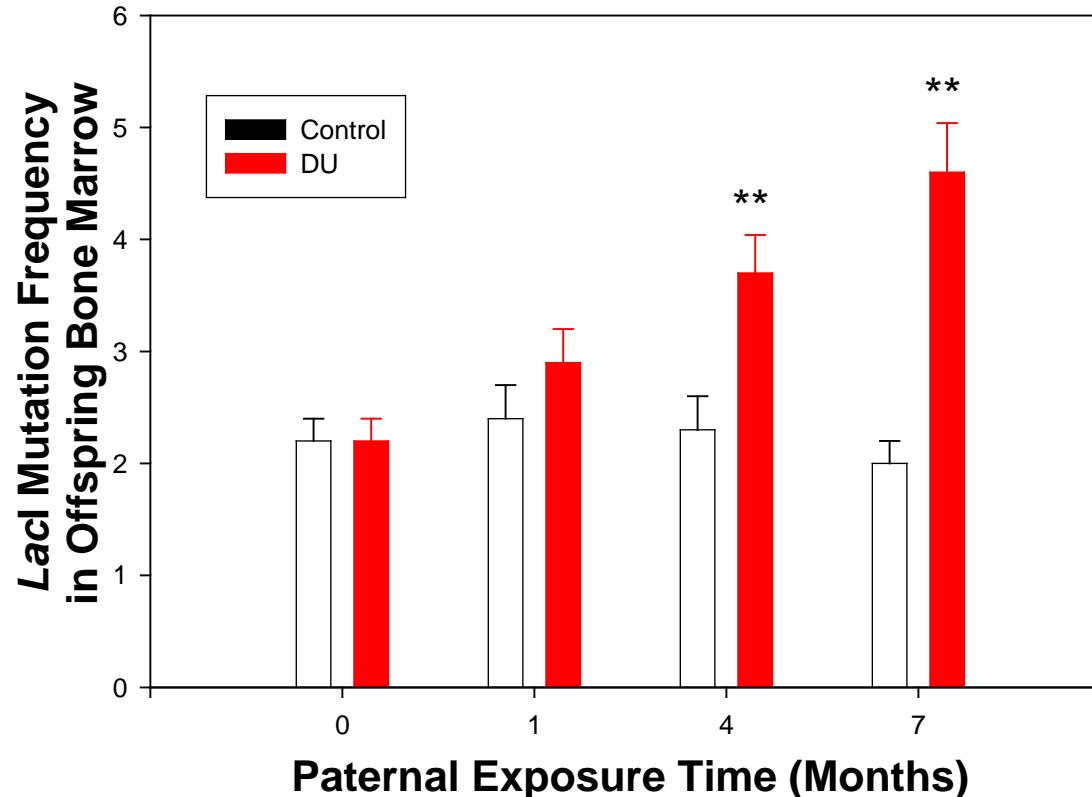


Transgenerational Effects of Depleted Uranium: Involvement of Radiation



Time-Dependence

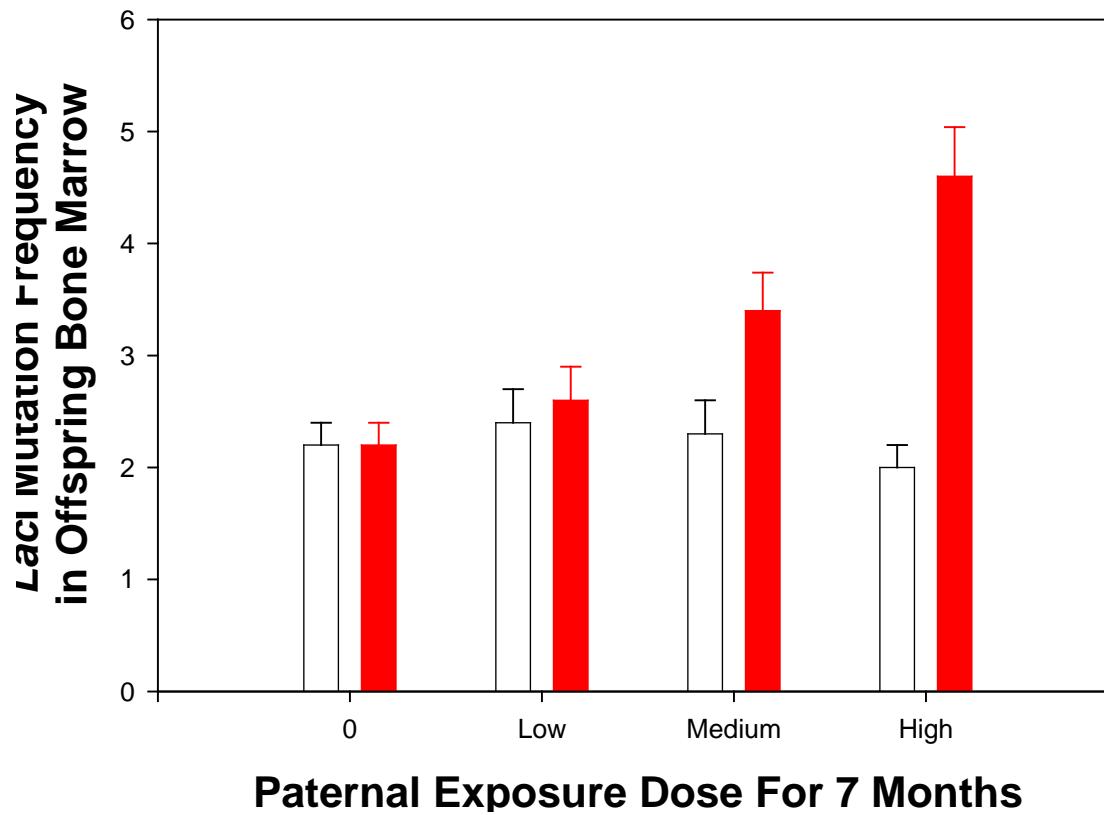
Mutation Frequencies in Bone Marrow of F1 Offspring Born to DU-implanted Male Mice (High dose)



*Studies were conducted with the support of the USAMRMC-CDMRP-PRMRP;
DAMD17-02-0185.*

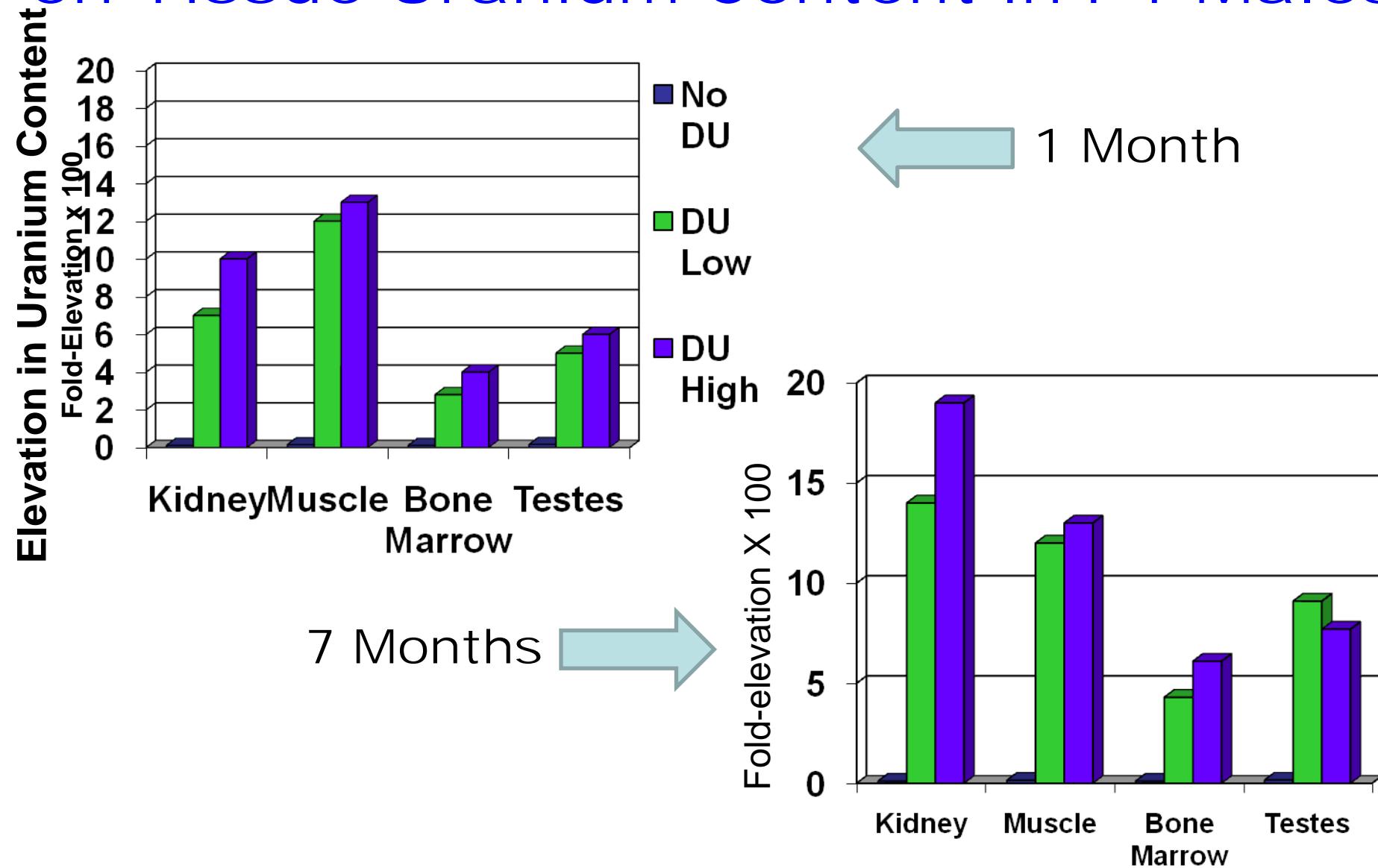
Dose-Dependence Transgenerational Effects *in Vivo*

Mutation Frequencies in Bone Marrow of F1 Offspring
Born to DU-implanted Male Mice (7 Months)

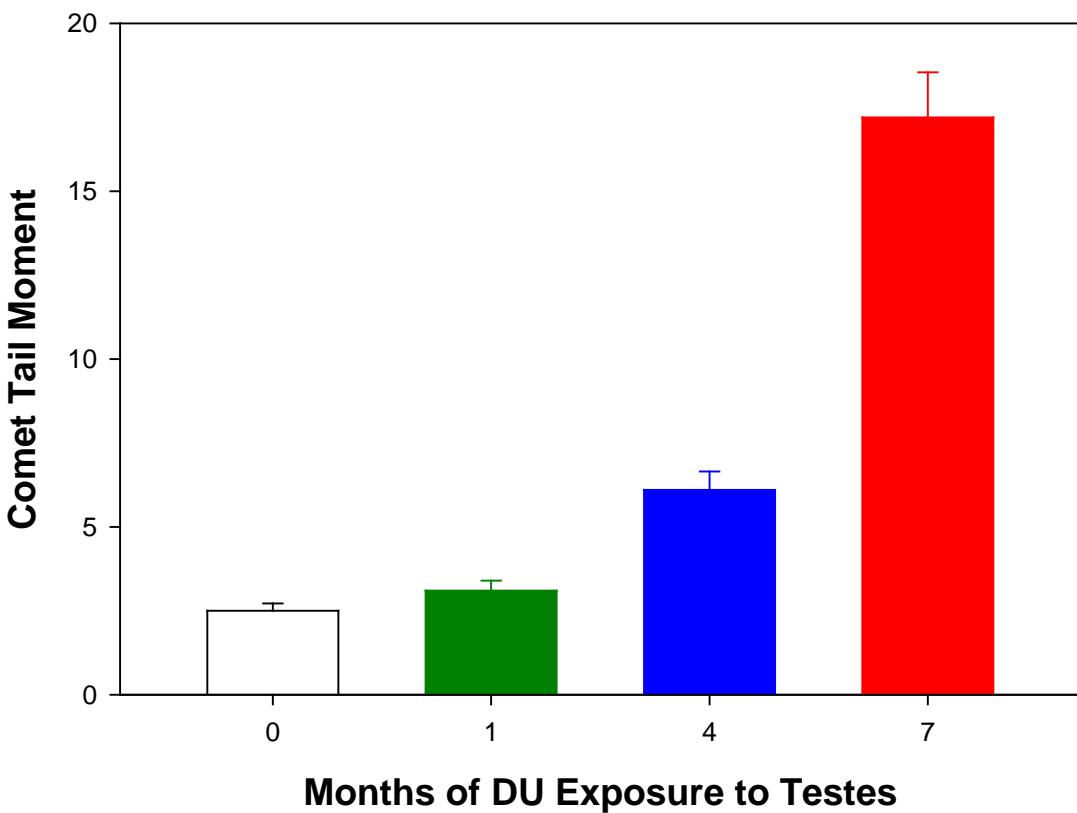


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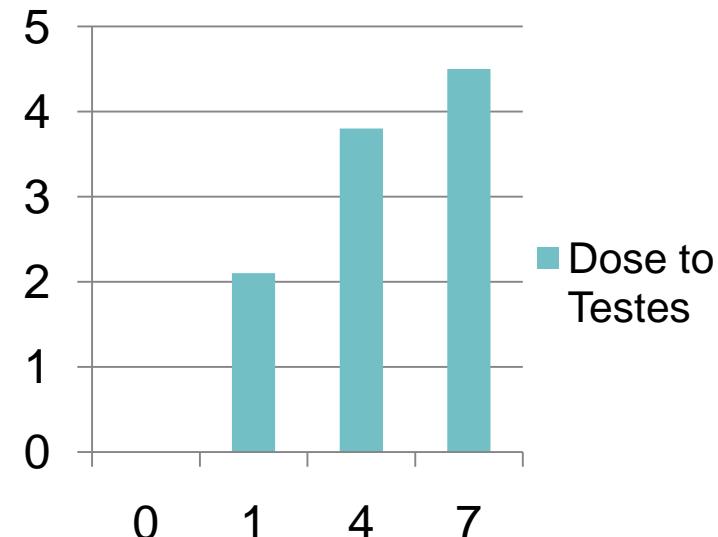
Effect of Internalized Exposure to DU on Tissue Uranium Content in P1 Males



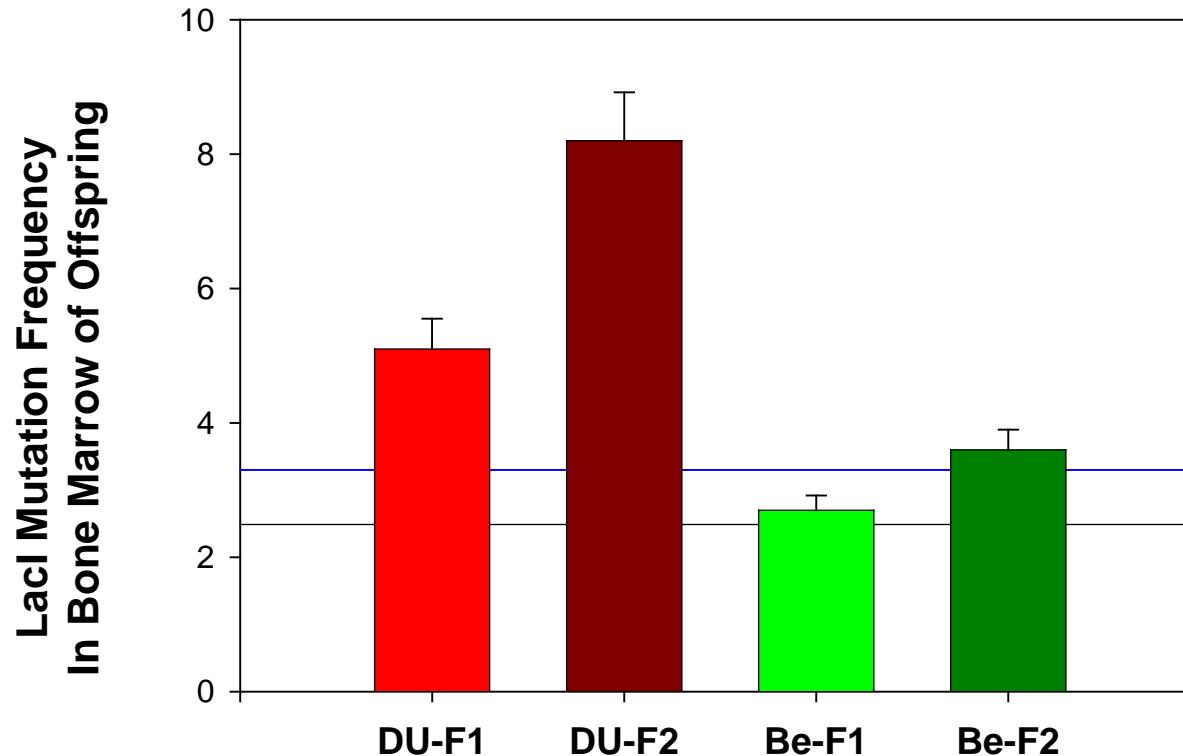
Germ Cell DNA Damage After Internalized DU Exposure in P1 Males



Preliminary Dose to Testes



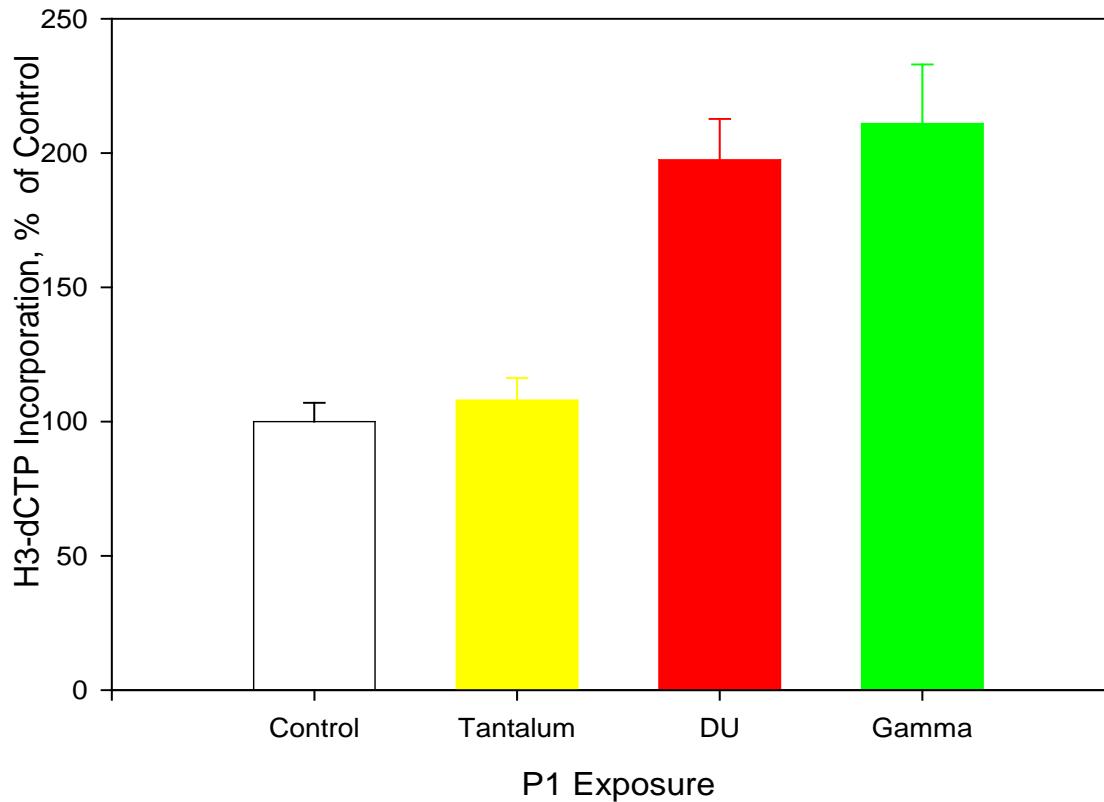
Mutation Frequencies in Bone Marrow of F2 Offspring Born to Heavy Metal-implanted Male Mice (High dose)



Lison, Monleau, Miller, in preparation

Epigenetic Mechanisms In Transgenerational DU Effects

Levels of DNA Methylation in Offspring Bone Marrow



Finding: Preconceptional Paternal DU Exposure Induces Genomic Instability in Unexposed Offspring

Finding: DU Internalized Exposure Induces Germ Cell DNA Damage

Evaluation of DU on Rat Behavior, Toxicological Endpoints, Male Reproduction

Uranium Distribution	Behavior Neurobio	Histopathology	Sperm Effects	Reproduction	Offspring Effects
Yes	No effects	No effects	No effects	No effects	F1 no cancers
Time and Dose Dependent					F1 ↑ Heart weight
					F1 unknown deaths assoc w P1 DU implants

Navy Medical detachment at Wright-Patterson Air Force Base

Arfsten DP, et al, 2005, Study of the reproductive effects in rats surgically implanted with depleted uranium for up to 90 days. *Toxicol Environ Health A.* Jun 11-25;68(11-12):967-97.

Arfsten DP, et al. 2006, Evaluation of the effect of implanted depleted uranium on male reproductive success, sperm concentration, and sperm velocity. *Environ Res.* Feb;100(2):205-15.

Arfsten DP, et al, 2007, Evaluation of the effect of implanted depleted uranium (DU) on adult rat behavior and toxicological endpoints. *J Toxicol Environ Health A.* 2007 Dec;70(23):1995-2010.

Arfsten DP, et al. 2009, Two-generation reproductive toxicity study of implanted depleted uranium (DU) in CD rats. *J Toxicol Environ Health A.* 72(6):410-27.

*Conclusions *in Vivo*:*

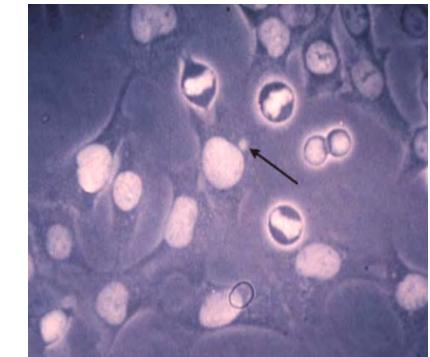
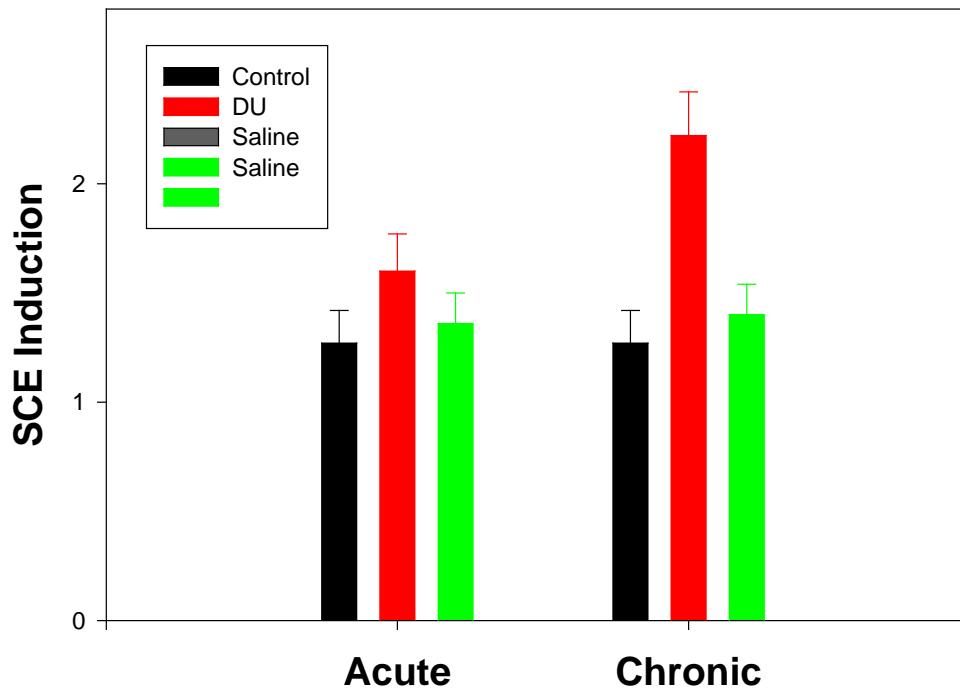
- 1. DU induces leukemia in a rodent model and is mutagenic *in vivo*.**
- 2. DU induces transgenerational genetic instability in unexposed rodent/mouse offspring. Radiation plays a role in this process.**
- 3. Reproductive toxicity has demonstrated conflicting results (oral versus embedded DU)**
- 4. DU Induces germ cell damage at high doses and long exposure times.**

Additional Preliminary Studies

- 1. Acute versus chronic**
- 2. Inhalation versus chronic embedded exposure**
- 3. Radiation effects *in vivo***

Acute Versus Chronic Uranium Exposure

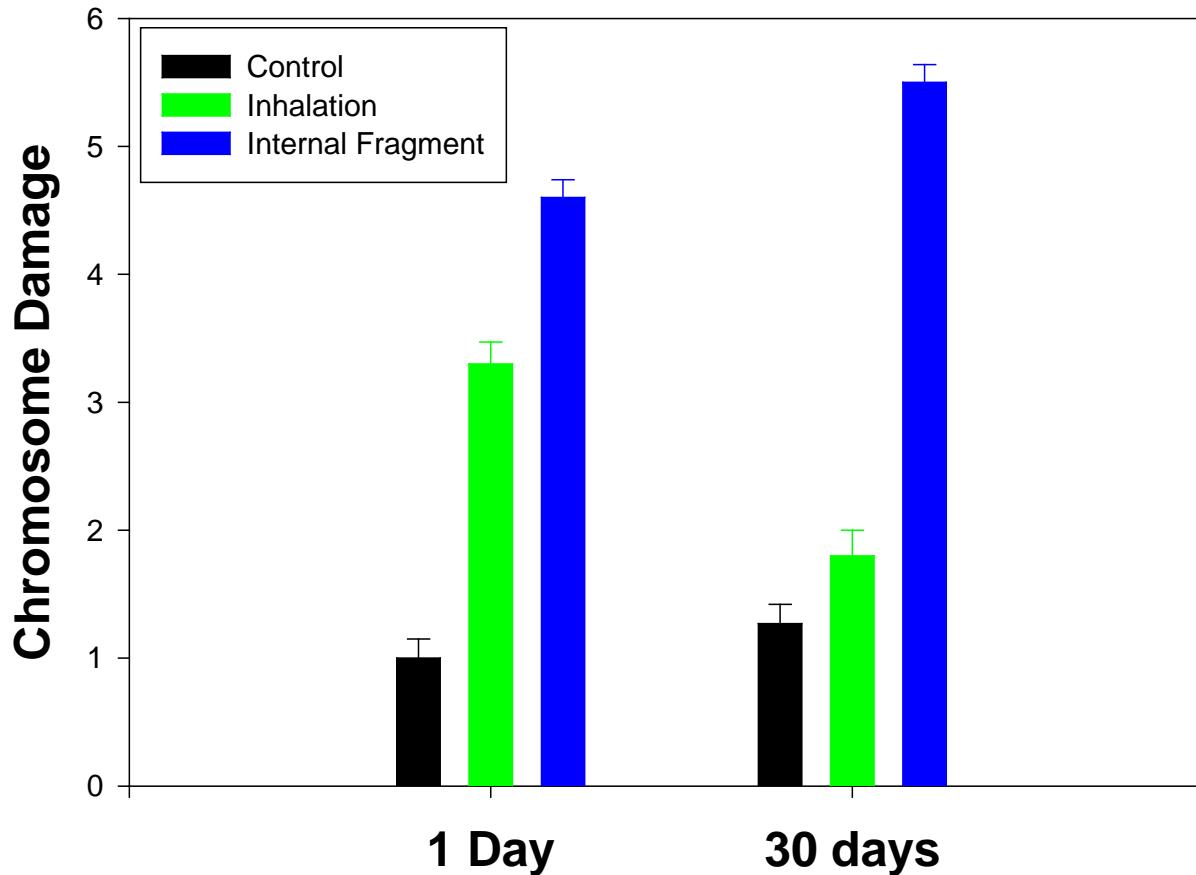
Comparison of Acute and Chronic DU Exposure: Induction of Micronuclei



Acute = 350 mg/kg DU (1 injection); 14 days post injection

Chronic = 35 mg/kg DU (1x daily, 10 days); 4 days post final injection

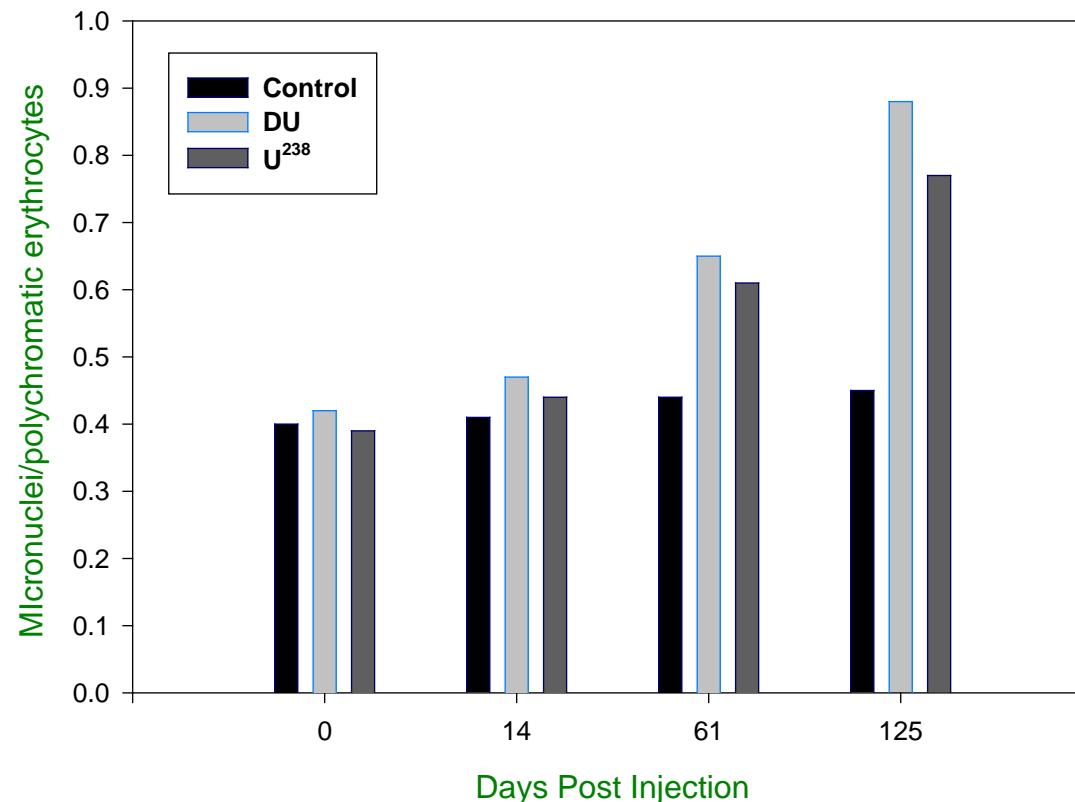
Comparison of Inhalation versus Chronic Internal Fragment Exposure *in vivo*: Measurement of Chromosomal Damage



Unpublished data

Radiation Effects *In Vivo*

Comparison of DU- and ^{238}U - Uranyl Nitrate *in vivo*:
Induction of Micronuclei

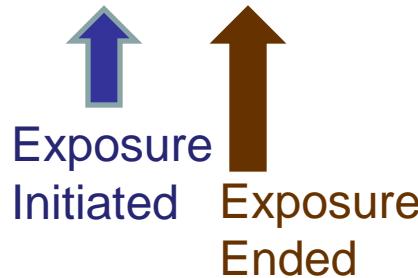
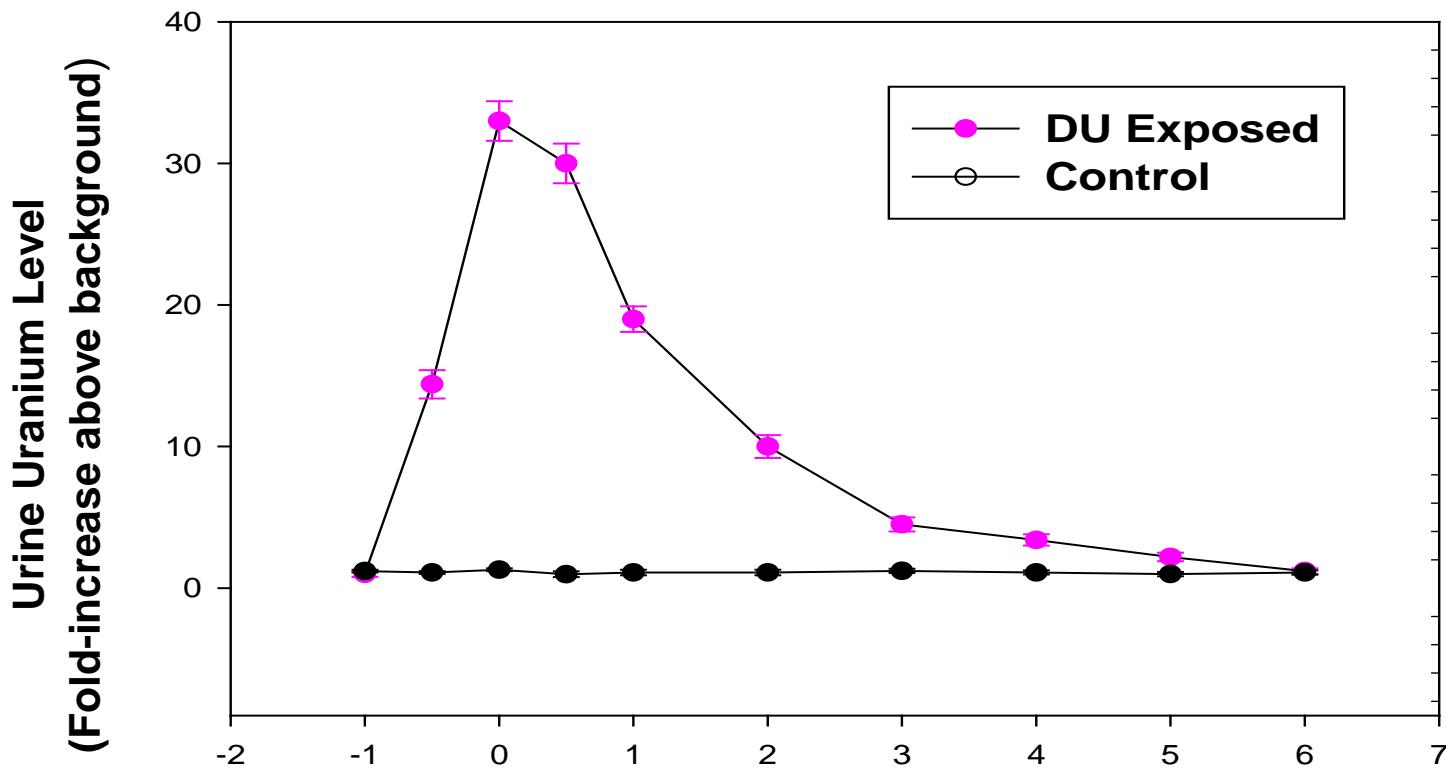


Unpublished data

Questions Regarding DU And Its Health and Biological Effects that Prompted Our Research

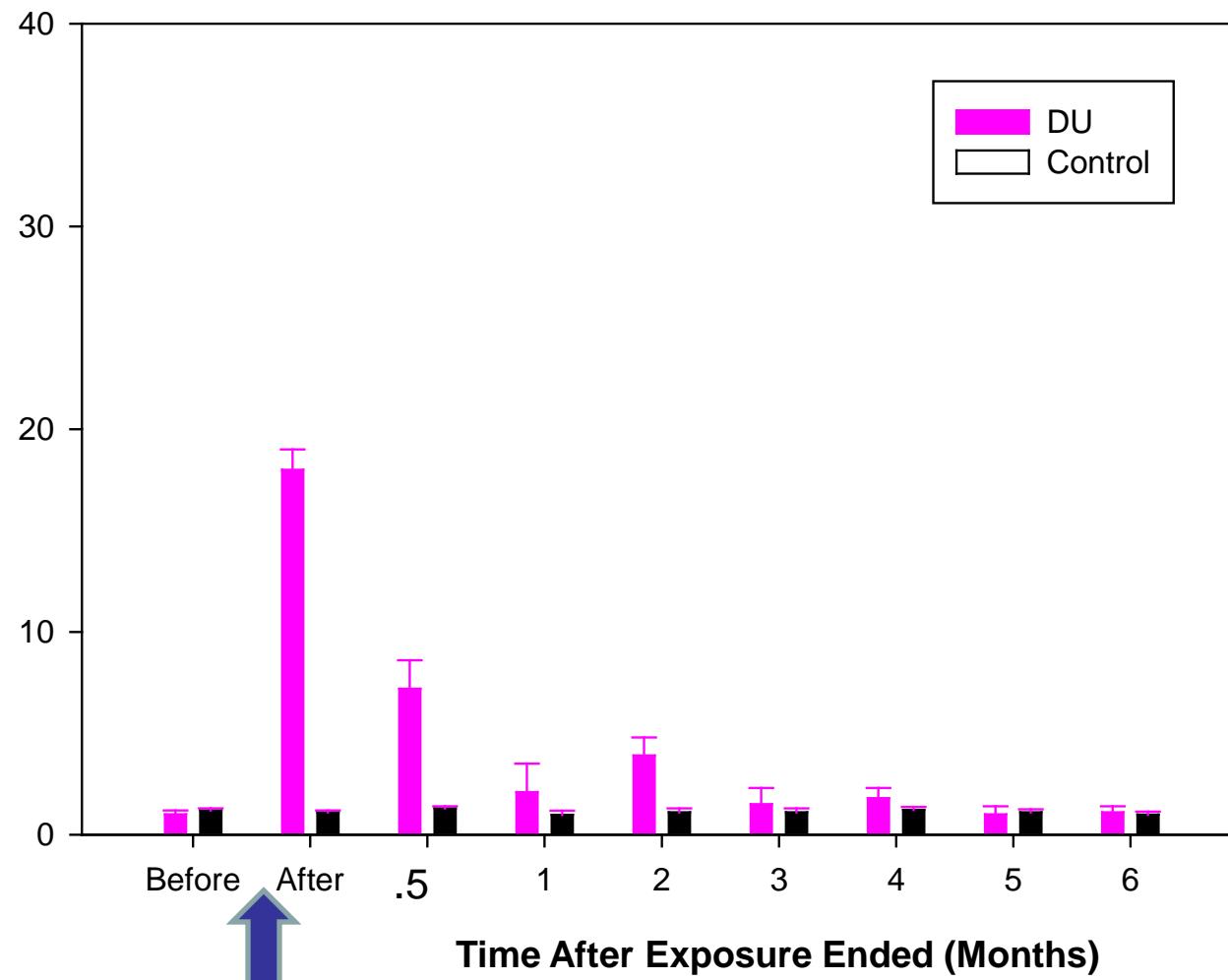
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Timecourse of Urine Uranium Content Following Internalized/Embedded DU Exposure



Time After Exposure Ended (Months)

Timecourse of Urine Uranium Content Following DU Inhalation

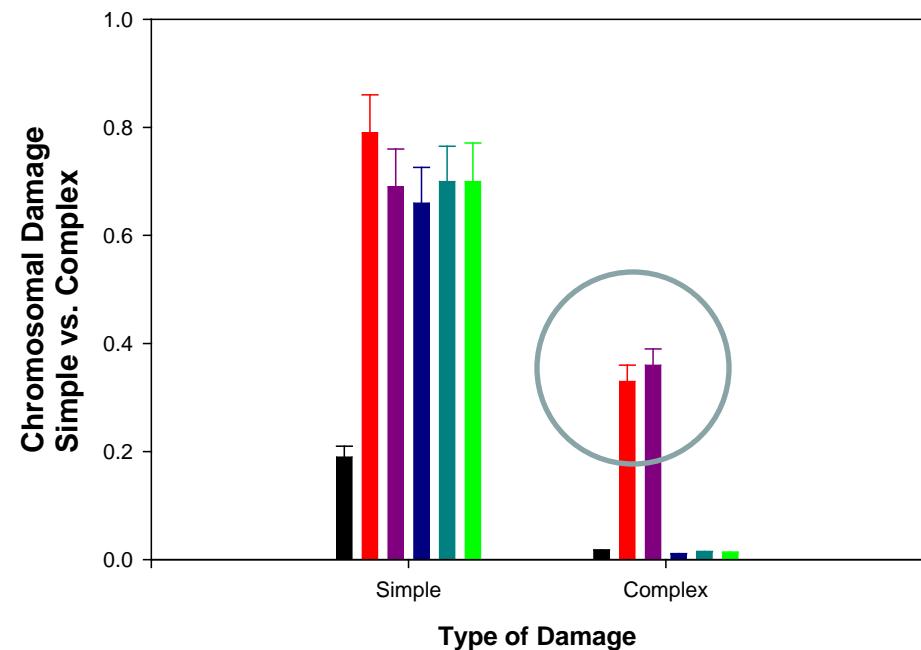
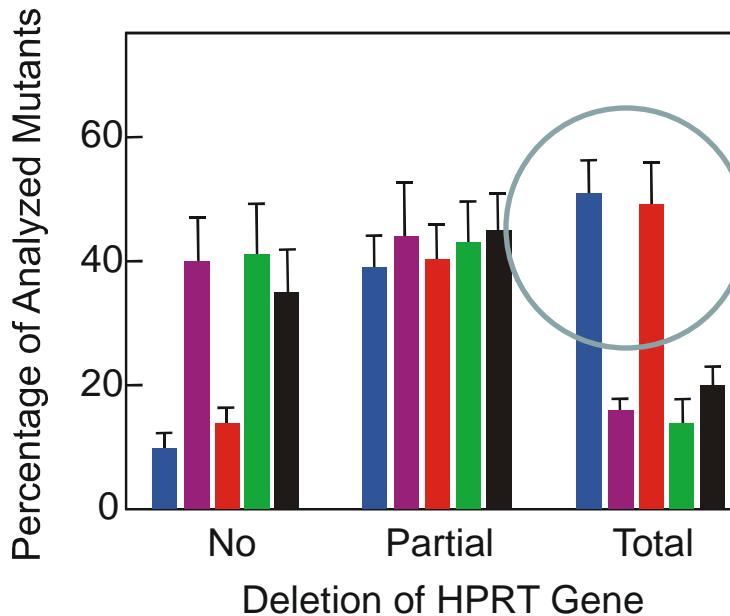


Is there a better exposure marker?

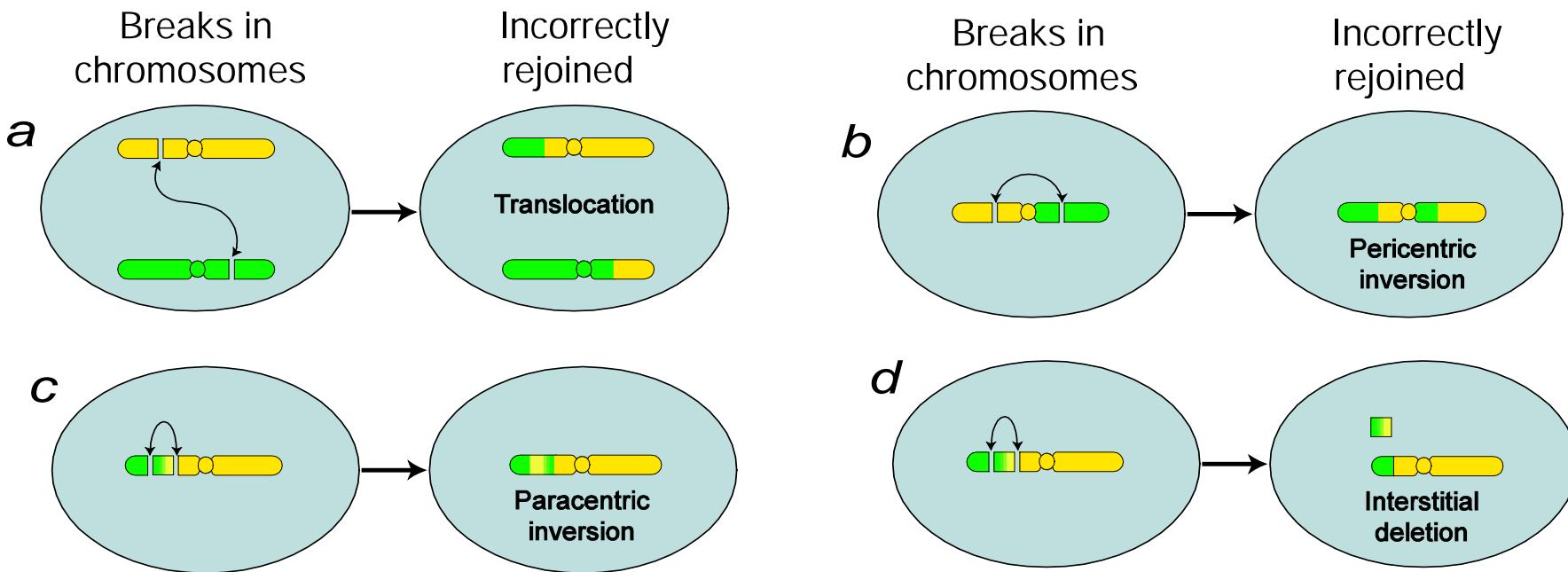
Good Marker Characteristics

- 1) Marker specificity**
- 2) Marker persistence**

DU and Alpha Particles Cause Similar Damage

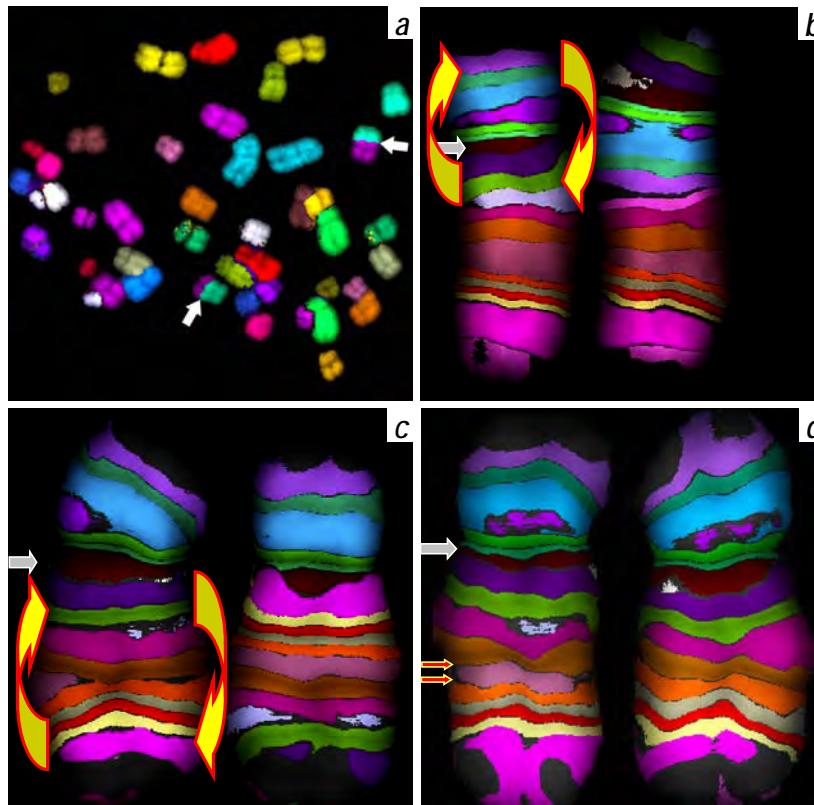


Chromosomal Damage as an Alpha Particle Exposure Biomarker



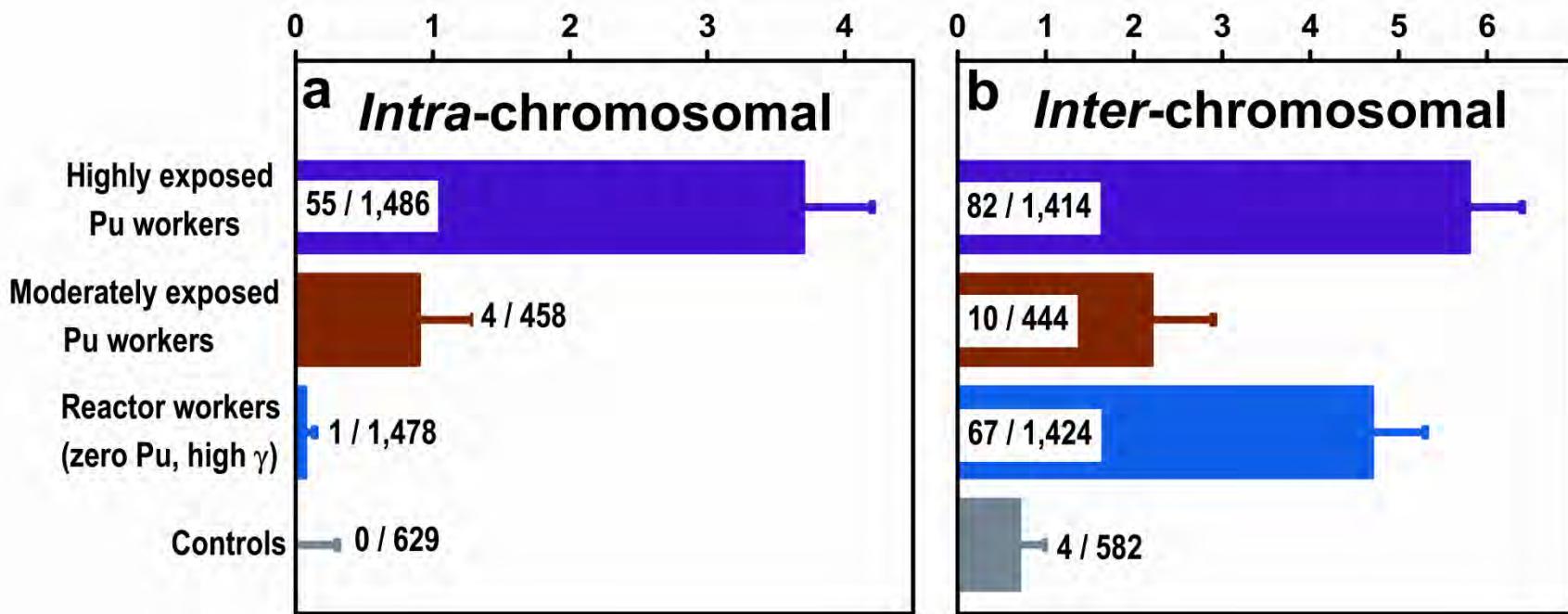
1. **(a)** inter-chromosomal aberration produced by misrejoining of chromosome breaks on two different chromosomes
2. **(b-d)** intra-chromosomal aberrations produced by misrejoining of breaks **(b)** on two different arms of a single chromosome, or **(c-d)** within a single chromosome arm. *Intra-chromosomal* aberrations generally originate from pairs of chromosome breaks that are closer together than those producing inter-chromosomal aberrations:

Development of Assay to Measure Stable Inter- and Intra- Chromosomal Aberrations in Workers Exposed to Alpha Particles Mayak Plutonium Workers



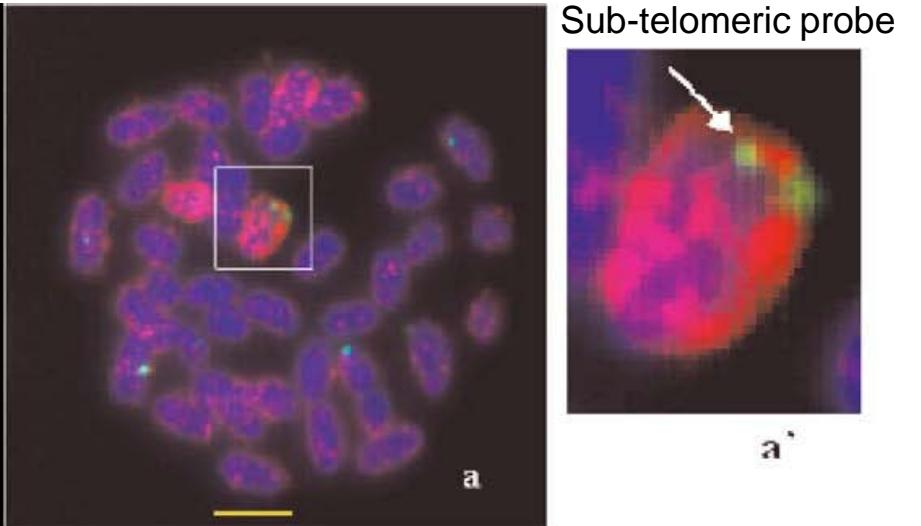
- a) Inter-chromosomal aberration (simple translocation),
- b) Inter-arm aberration (pericentric inversion)
- c) Intra-chromosomal intra-arm aberration (paracentric inversion)
- d) Intra-chromosomal intra-arm aberration (intra-arm deletion)

Measured Yields of Stable Chromosomal Aberrations In Peripheral Blood Lymphocytes Of Mayak Workers

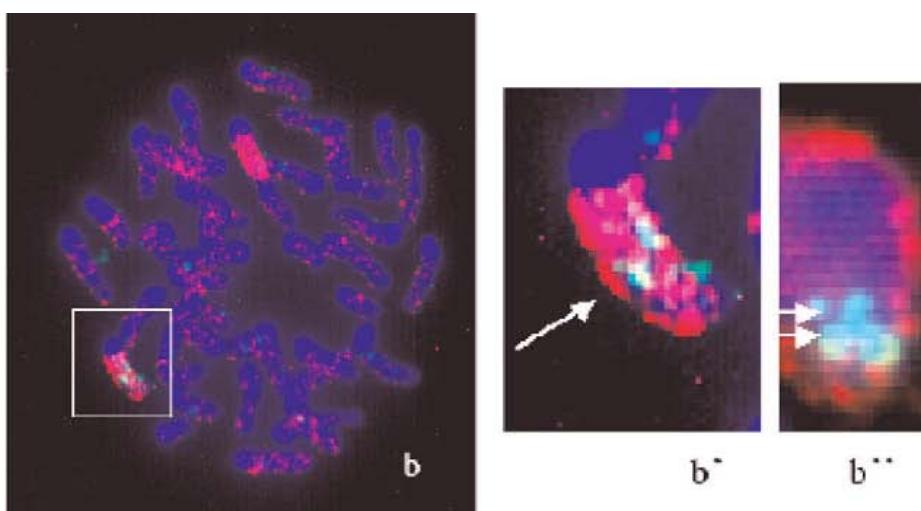


Need a large number of samples

Instability of Mouse Chromosome 11: Rearrangement of the telomeric region in Mouse Samples



Exposure	Insert Chr 11	Translo Chr 11	Invers Chr 11
DU	16/100	10/100	10/100
^{60}Co Gamma	5/100	5/100	0
Tungsten alloy	6/100	6/100	0
Ethyl benzene	4/100	8/100	0



**FISH with a subtelomeric probe for
Mouse chromosome 11.**

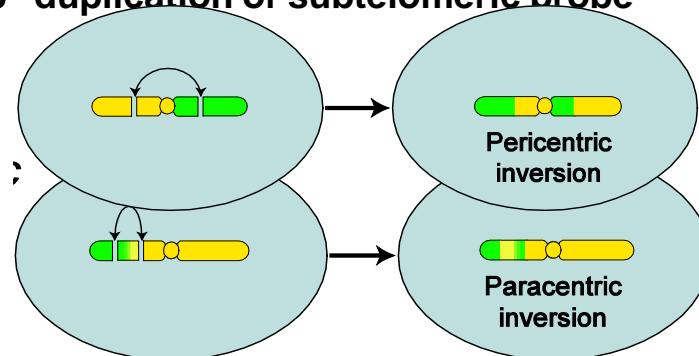
a Metaphase normal chr 11

a' enlargement of Chr 11; 2 subtelomeric signals

b elongated chr; inverted duplication

b' green probe seen as doublet

b'' duplication of subtelomeric probe



*Conclusions *in Vivo*:*

Internalized chronic DU exposure *in vivo*:

- 1. - causes uranium re-distribution to multiple organs.**
- 2. - Is associated with urine mutagenicity**
- 3. - Induces chromosomal damage**
- 4. - Indices leukemia development in mice**
- 5. - causes preconceptional paternal exposure to induce genomic damage in unexposed offspring**
- 6. - Induces germ cell DNA damage**

These results indicate that the responses are DU dose- and time- dependent

AFRRI – My Lab

Stuart Cohen

Rafael Rivas

Karvi Miller

Mike Stewart

AFRRI

Chris Lissner

John Gilstad

John Kalinich

Bill Blakely

Terry Pellmar

PGS Prasanna

Dave McClain

David Livengood

Henry Gerstenberg

John Ejnik

Ted St. John

Shelly Hodge

Christy Emond

Tom Dalton

Jessica Kordell

Vernieda Vergara

Danny Beltran

Tim Whittaker

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Kia Brooks

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Lei Luo

Natalie Page

John Chen

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Mark Hill

Rona Anderson

University Of Paris

Paul Lison

Robert Merlot

Catherine Bonait-Pellie

Jean Michel

Lillian Crepin

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French Institute

Nuclear Security

Francois Paquet

Valerie Chavel

Pascal Houpert

Henri Metivier

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CAPT Young, USA
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SGT Tyson, USA

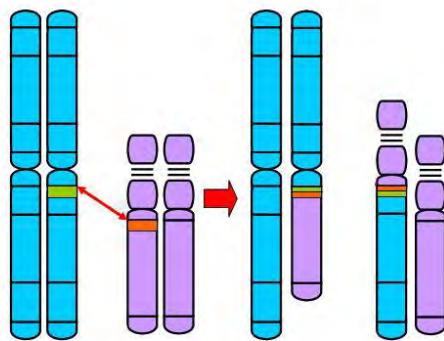
AFRRI/USU

Donna SOLYAN
David Morse
Frank Duffy
Yvonne Smith
Patrice Bolte
Linda Culp
Catherine Crennan

AFRRI 50th Committee

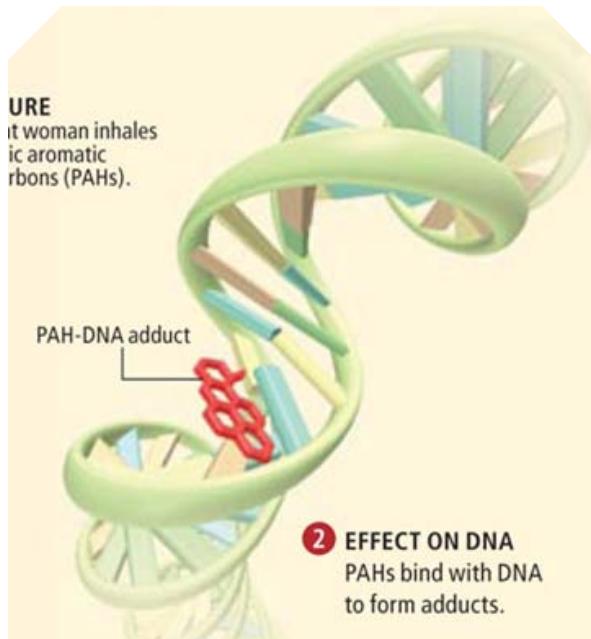
Mark Melanson, COL, USA
John Gilstad, CAPT, USN
Chris Lissner, CAPT, USN (Ret)
Eric Lombardini, MAJ, USA
Mike Dempsey, COL, USA
Donna Solyan
William Blakely
David Ledney
Mike Landauer
Mark Whitnall
Alexandra Miller

Other Biomarkers

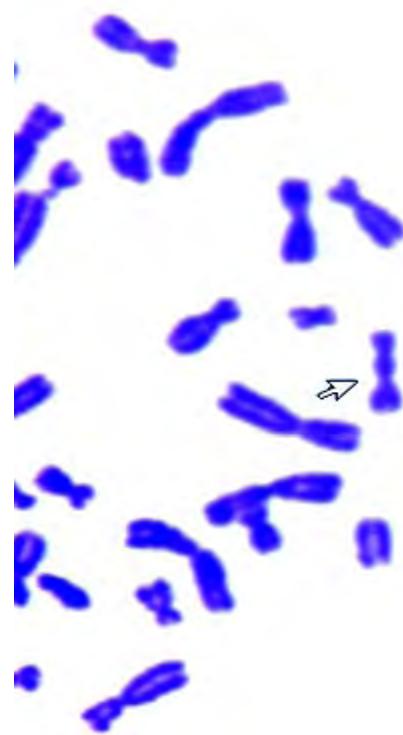


Translocations

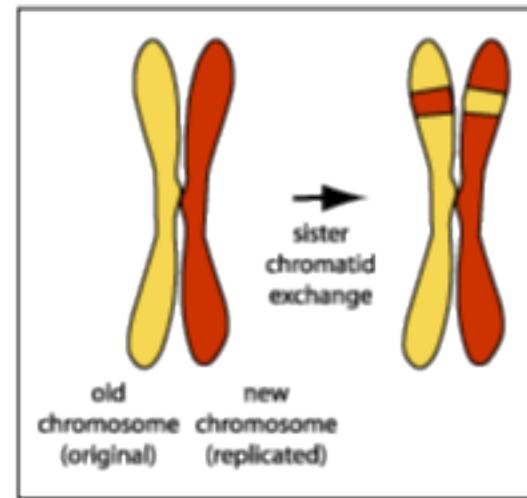
DNA Adducts



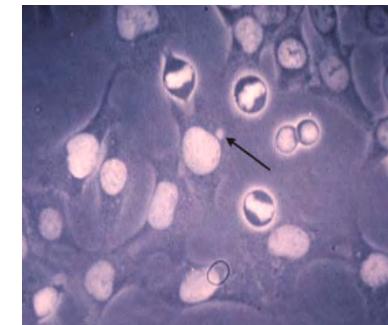
Dicentrics



Sister Chromatid Exchange



Micronuclei

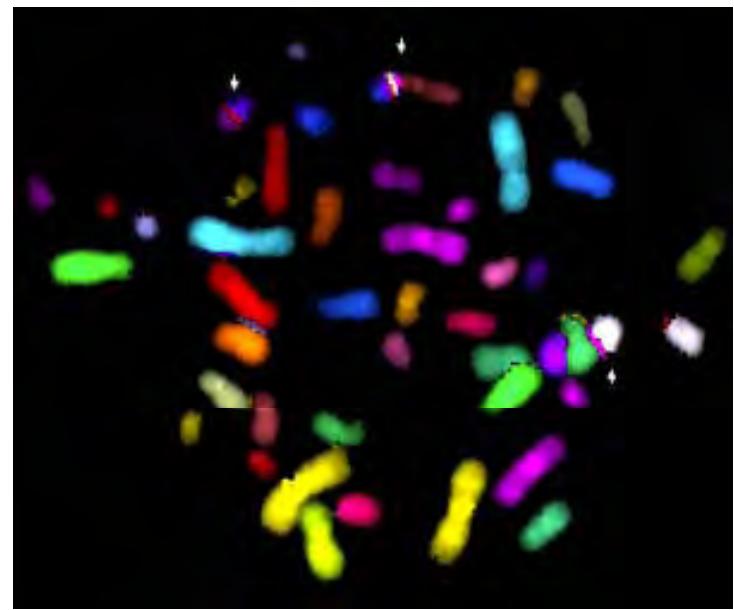
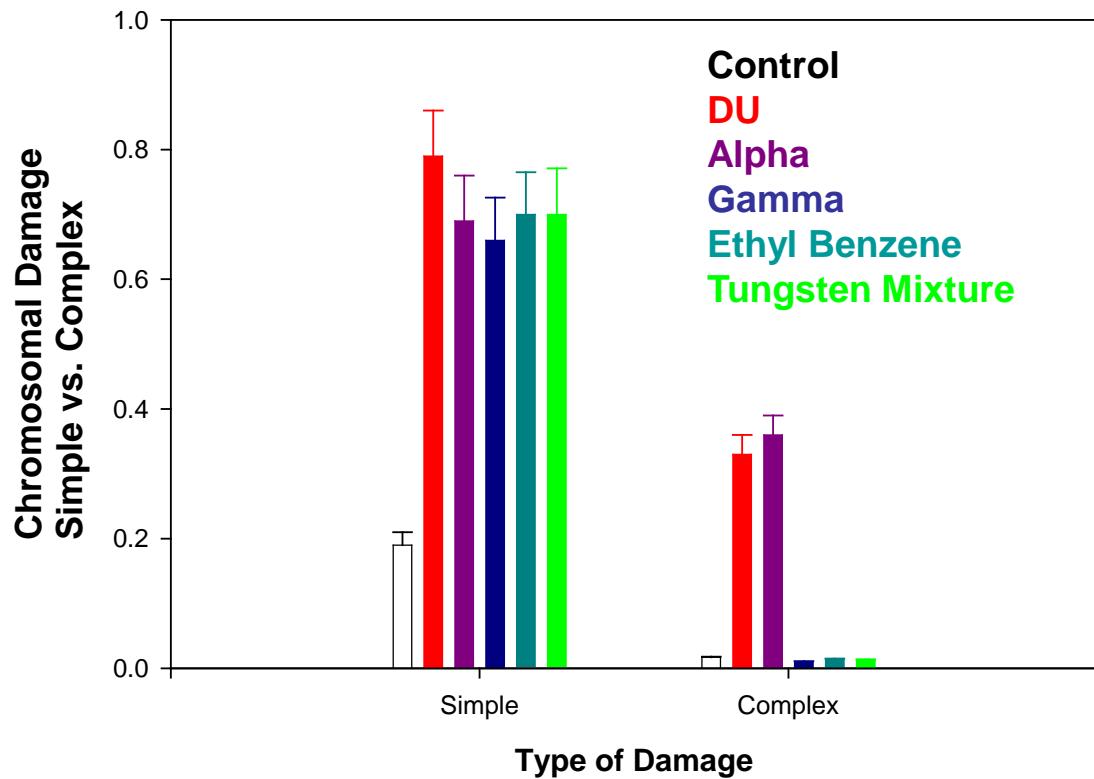


Simple vs Complex Damage

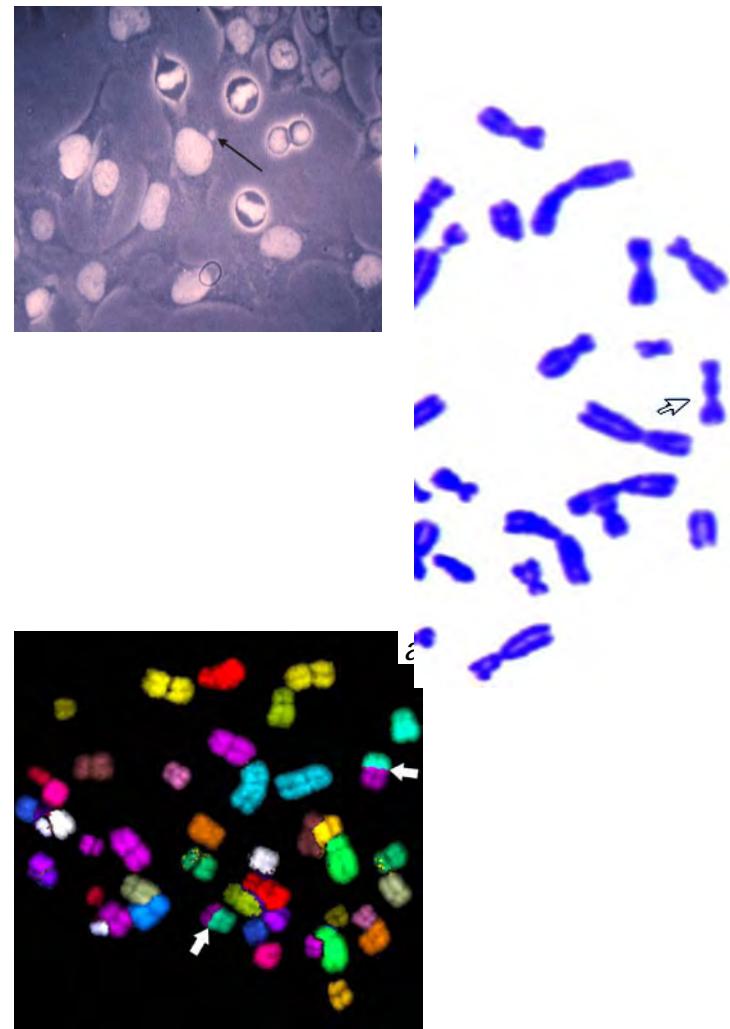
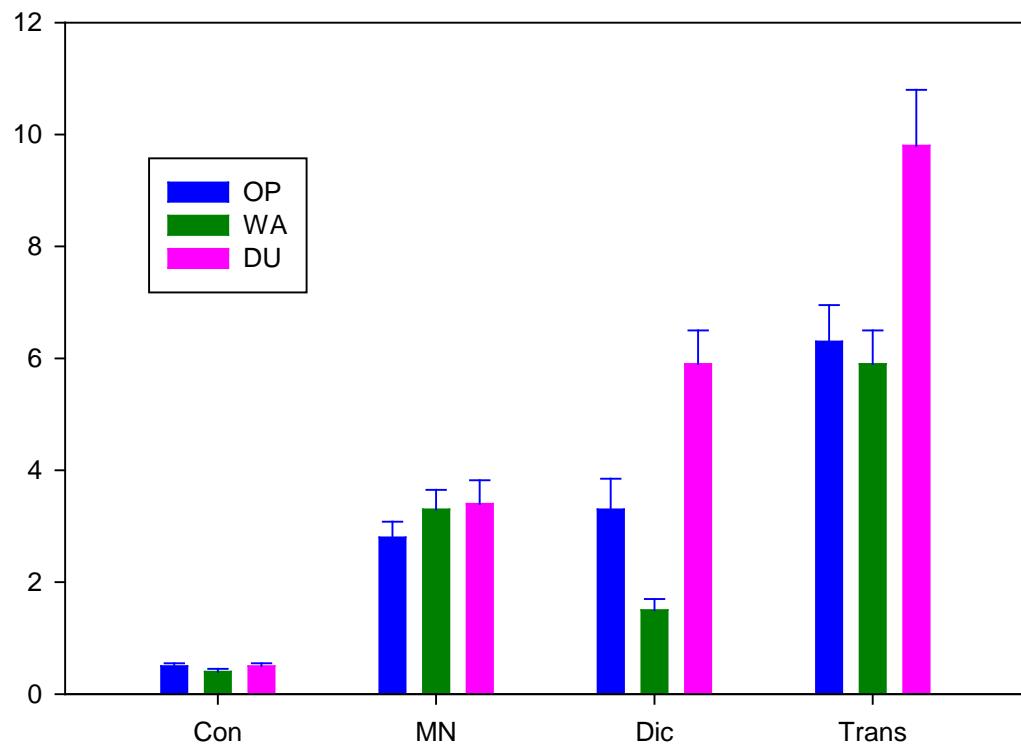
Simple: 1 or 2 breaks in 1 chromosome

Complex: 3 or more breaks in 2 or more chromosomes

Chromosomal Damage in Human Osteoblast Cells Exposed to DU, Alpha Particles, Gamma Radiation, Tungsten Mixture, Ethyl Benzene *in vitro*

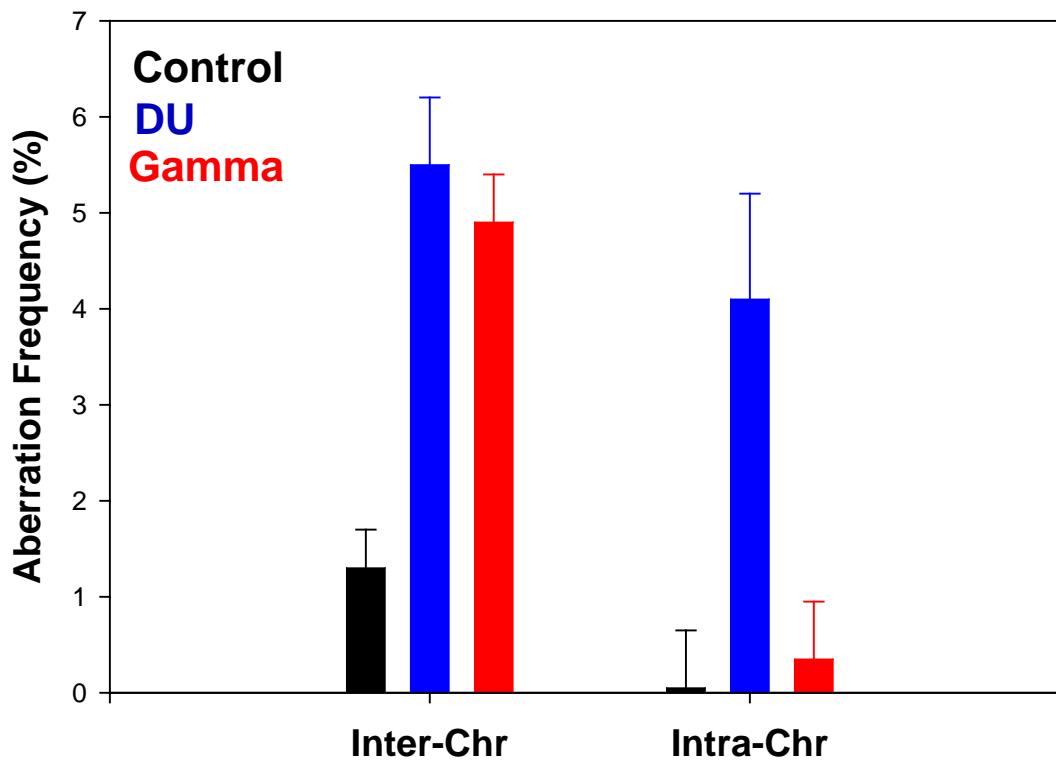


Early Cytogenetic Damage in HOS Cells: Organophosphate, Tungsten Alloy, Depleted Uranium



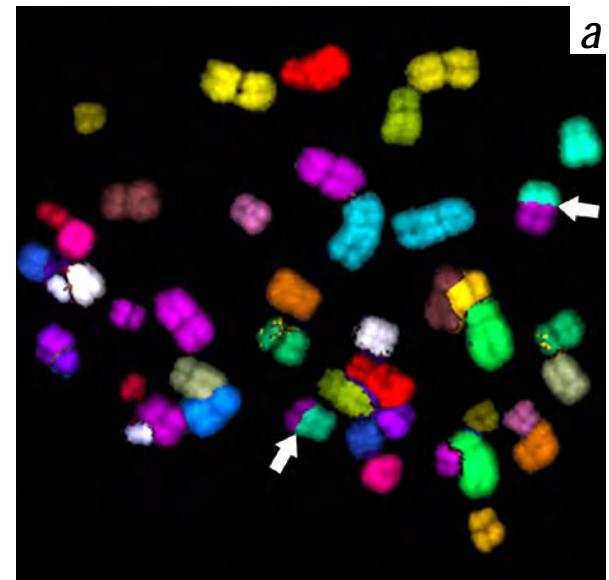
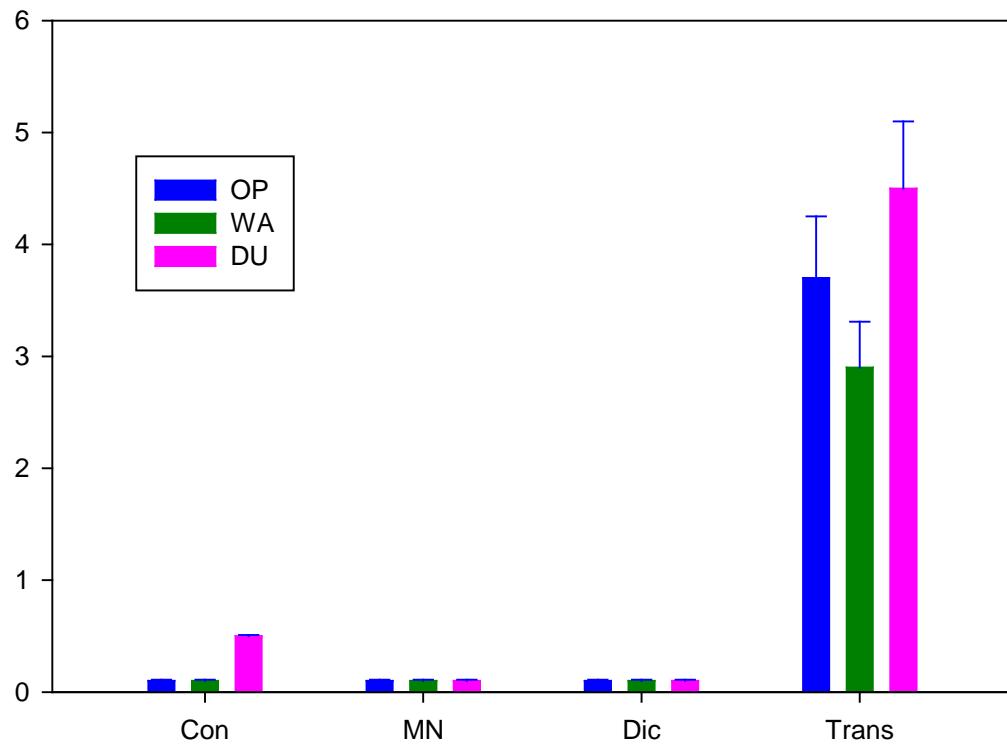
mBAND Assay

Measured Yields of Stable Chromosomal Aberrations *in vivo*



Persistence and Specificity

Persistent Cytogenetic Damage in HOS Cells: Organophosphate, Tungsten Alloy, Depleted Uranium



Chromosomal Translocations are similar